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(71) Applicants *(for all designated States except US)*: **TRUSTEES OF BOSTON UNIVERSITY** [US/US]; One Sherborn Street, Boston, MA 02215 (US). **CELLICON BIOTECHNOLOGIES, INC.** [US/US]; 29 Linden Square, Wellesley, MA 02482 (US). **PURETECH VENTURES** [US/US]; 222 Berkeley Street, Suite 1040, Boston, MA 02116 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **COTTAREL, Guillaume** [FR/US]; 4 Freeman Circle, Lexington, MA 02421 (US). **WIERZBOWSKI, Jamey** [US/US]; 35 Katherine Road, Stoneham, MA 02180 (US). **PAL, Kollol** [US/US]; 205 Tudor Road, Needham, MA 02492 (US). **KOHANSKI, Michael** [US/US]; 15 N Beacon Street, Apt. 1002, Allston, MA 02134 (US). **DWYER, Daniel** [US/US]; 15 Rangeley Road, Watertown, MA 02472 (US). **COLLINS, James** [US/US]; One Sherborn Street (US). **ALMSTETTER, Michael** [DE/DE]; Winklerring 29, D-85630 Grasbrunn (DE). **THORMANN, Michael** [DE/DE]; Kopernikusweg 1, D-82152 Martinsried (DE). **TREML, Andreas** [DE/DE]; Bermeister Stolzl Str. 11, 94249 Bodenmais (DE).

(74) Agent: JARRELL, Brenda, Herschbach; Choate, Hall & Stewart, Two International Place, Boston, MA 02110 (US).

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(54) Title: COMPOSITIONS AND METHODS FOR ANTIBIOTIC POTENTIATION AND DRUG DISCOVERS

(57) Abstract: The present invention provides methods for identifying target genes whose partial or complete functional inactivation potentiates the activity of an antibiotic agent, e.g., a quinolone antibiotic. The invention further provides methods for identifying agents that modulate expression of target genes or that modulate activity of expression products of target genes. Agents identified according to various methods of the invention potentiate the activity of antibiotics such as quinolones, aminoglycosides, peptide antibiotics and β-lactams. Also provided are agents that suppress and/or retard resistance to antibiotics. The inventive methods provide potentiating agents and compositions comprising potentiating agents and antibiotics. Such agents and compositions can be used for inhibiting growth or survival of a microbial cell or of treating a subject suffering from or susceptible to a microbial infection.

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Compositions and Methods for Antibiotic Potentiation and Drug Discovery

Related Applications

[0001] The present application claims priority from U.S. Provisional Application No. 60/772,648 filed on February 13, 2006 and entitled "Compositions and Methods for Antibiotic Potentiation and Drug Discovery", and from U.S. Provisional Application No. 60/835,596 filed on August 4, 2006 and entitled "Rec A Inhibition". Each of the provisional applications is incorporated herein by reference in its entirety.

Background of the Invention

[0002] The emergence of resistance to antibacterial agents is a growing problem for human and animal health, and new drugs to treat infections due to microorganisms that display resistance to currently used antibiotics are urgently needed. Efforts to overcome the growing problem of resistance have included modification of known antibiotics, classical screening of new compound libraries and natural product libraries, and genomic efforts to identify novel targets to which no cross resistance with existing antibiotics would be anticipated. Even with this significant antibiotic discovery effort, only a few agents that represent new chemical classes of antibiotic agents have been approved by regulatory agencies in recent years. In addition, few antibiotics that are effective against bacterial that have developed resistance to currently used antibiotics are in clinical development. Furthermore, a number of potent antibiotic agents are too toxic for clinical use or have significant side effects that limit their therapeutic utility.

[0003] Quinolones are one of the most widely used classes of antimicrobial agents worldwide and serve to exemplify the promise and the problems associated with antibiotic development and use. Their therapeutic indications in humans have evolved from urinary tract infections to infections of almost all body compartments. Various members of the class are also widely administered for veterinary purposes.

[0004] Quinolones exert their antimicrobial effect by targeting bacterial type II topoisomerases, namely gyrase and topoisomerase IV, essential enzymes that catalyze breakage and rejoining of DNA strands during normal cell growth. Quinolones form a ternary complex between DNA and either gyrase or topoisomerase IV, thereby blocking DNA replication and leading to events such as double-stranded DNA breaks that are rapidly lethal to the cell. As a class, quinolones have a broad spectrum of activity against Gram positive and Gram negative species, including both aerobic and anaerobic microorganisms. Activity against Gram negative microorganisms often depends strongly upon activity against DNA gyrase, while topoisomerase IV is often the primary target of quinolones that display activity against Gram positive species. A number of therapeutically useful quinolones display activity towards both of these targets.

[0005] Quinolones, like most other antibiotics, can have adverse effects with the potential to affect virtually every major system in the body. The most frequent side effects associated with fluoroquinolone use involve the GI tract, skin, and/or central nervous system (CNS). Fluoroquinolones can also cause hypersensitivity reactions. A mild transient increase in liver function is seen in 2-3 % of the patients who are treated with a fluoroquinolone, and severe liver toxicity is a rare side effect of quinolone use. Cardiac side effects such as prolongation of the QT interval, which may predispose to serious arrhythmias, is also a concern.

[0006] The adverse effects of many potent quinolones has hindered efforts to develop them as therapeutic agents. Gemifloxacin, for example, has an enhanced affinity for its target relative to many other quinolones and displays potent activity against most Gram positive cocci, particularly against *Streptomyces pneumoniae.* Gemifloxacin is 30 fold more active than ciprofloxacin against this pathogen. Unfortunately, the side effects of the compound were significant enough that the further development by the pharmaceutical company was terminated.

[0007] In addition, the rapid development of resistance to a number of quinolones is a cause for alarm and has led to a number of treatment failures. The spread of resistance has motivated calls for the development of policies that would restrict unnecessary use of these agents. Resistance can develop in a variety of ways including mutations in the genes encoding either DNA gyrase or topoisomerase IV and overexpression of efflux pumps.

[0008] Quinolones are only one example of antibiotic agents that exhibit side effects and/or to which resistance has been developed. Thus, there is clearly a need in the art both for new agents to combat microbial infection and for new approaches to antibiotic drug discovery. There is also a need in the art for new approaches to improving the safety profile of certain antibiotics.

Summary of the Invention

[0009] The present invention provides compositions and methods for potentiating the activity of antibiotic agents and for discovering new agents of use in treating bacterial infection.

[0010] In one aspect, the invention provides a method of inhibiting growth or survival of a microbial cell comprising: (a) contacting the microbial cell with a antibiotic (*e.g.*, an quinolone); and (b) contacting the cell with an agent that potentiates activity of the antibiotic.

[0011] In another aspect the invention provides a method of treating a subject in need thereof comprising: administering an antibiotic to the subject in combination with an agent that potentiates activity of the antibiotic. In certain embodiments of either of the foregoing methods, the agent preferably inhibits or interferes with activity of an expression product of an antibiotic potentiator target gene. In either of the methods the agent and the antibiotic may be administered individually or as components of a single composition. The antibiotic may be used at a concentration below its MIC, or may be administered at a dose below that at which it would be effective as a single agent.

[0012] In another aspect, the invention provides a method of identifying an antibiotic potentiator target gene comprising steps of: (a) contacting a microbial cell with an antibiotic, wherein the microbial cell has a genetic alteration that significantly reduces or eliminates expression of a gene; (b) comparing growth or survival of the microbial cell with growth or survival of a microbial cell having higher expression of the gene than the cell having the genetic alteration; and (c) determining that the gene is an antibiotic potentiator target gene if the growth, survival, or both of the microbial cell having the genetic alteration is lower than the growth, survival, or both of the microbial cell having higher expression of the gene.

[0013] In another aspect the invention provides a set of target genes whose functional inactivation potentiates the activity of antibiotics. The set comprises *dapF, fabH, fis, fliN, jw5303, priA, recA, recB, recC, recG, resA, ruvA, ruvB, ruvC, ruvC, uvrD, ycjS,ydfL, yhfT, yqgC, aceE, araJ, aspA, atpA, atpF, atpH, brnQ, cydB, degP, dnaT, fepD, folP, guaB, Hfq, iscS, JW5360, lipA, lpdA, nuoB, nuoJ, nuoL, osmB, pdxH, pnuC, priA, priB, rbfA, recA, rfaD, rpmJ, rpoD, resA, ruvC, sdhC, seqA, setA, sucB, surA, tar, tatE, tauB, trmU, ubiH, ubiX, xerC, yaaU, ycfM, yejO, ygfZ, ygiH, yheL, yheM, yheN, yicG, yjdL, yjeT, yjiO, yjjY, agaAk, atpA, atpF, atpH, bglF, cysE, cysI, fepC, fepD, frvR, guaA, guaB, hofF, hsdS, iscS,*

JW4016, JW5075, JW5227, JW5257, JW5360, kdgK, lipA, lysA, malG, mbhA, mdoG, Nei, nmpC, nudH, pdxH, phnB, phnL, phnO, pnuC, potE, pshM, ptsA, rhaT, rpiA, resA, Sbp, speA, sucB, sugE, tdcE, tdcG, tolC, trxA, ubiE, ubiH, ubiX, Xni, ybbY, ycfM, ydeJ, yeeY, yfeT, ygaA, ygfZ, yhdX, yheL, yheM, yiaY, yidK, yihV, yjbN, yjcR, yjcZ, ynjD, yqeC, yqiH, and *yrfA*.

[0014] In another aspect the invention provides an assay system comprising: (a) a test agent; and (b) components suitable for performing an assay that detects expression of an antibiotic potentiator target gene or detects activity of an expression product of an antibiotic potentiator target gene, wherein the gene is from the set described above.

[0015] The invention further provides methods of identifying an agent that modulates expression and/or activity of an antibiotic potentiator target gene or its expression product.

[0016] One such method comprises (a) providing an assay system comprising a test compound, wherein the assay system is suitable for performing an assay to detect expression of an antibiotic potentiator target gene or to detect activity of an expression product of an antibiotic potentiator target gene; and (b) determining whether the test compound inhibits expression of the antibiotic potentiator target gene or inhibits or interferes with activity of an expression product of the gene; and (c) identifying the test compound as a potentiator of the antibiotic if the test agent inhibits expression of the potentiator target gene or inhibits or interferes with activity of an expression product of the potentiator target gene.

[0017] A variety of assays suitable for identifying agents that inhibit polypeptides encoded by specific target genes, e.g., RecA, are provided. Assays provided include binding assays, reporter-based assays, and enzymatic assays. Also provided are collections of bacterial strains suitable for performing the target gene identification methods of the invention and/or for identifying agents that modulate expression of a target gene or modulate activity of an expression product of a target gene.

[0018] In another aspect, the invention provides a method of identifying an agent comprising steps of: (a) computationally analyzing interaction of each of a plurality of test agents with a protein comprising a polypeptide encoded by an antibiotic potentiator target gene with each of a plurality of test agents; and (b) identifying at least one test agent having favorable interactions with the active site. The agent may be further tested, e.g., to determine whether it potentiates activity of an antibiotic against bacteria.

[0019] In another aspect the invention provides a composition comprising an agent (potentiating agent) that inhibits activity of an expression product of an antibiotic potentiator target gene. In another aspect the invention provides a composition comprising an inhibitor of a microbial type II topoisomerase and an agent that inhibits activity of an expression product of a quinolone potentiator target gene. In certain embodiments of the invention the potentiating compound is active by itself as an antibiotic. In other embodiments of the invention the potentiating compound is not active by itself as an antibiotic (at doses that can be tolerated in accordance with sound medical practice). The agent modulates expression of an antibiotic potentiator target gene or activity of an expression product of the target gene. The agent may, for example, (i) inhibit or interfere with expression of a microbial antibiotic potentiator target gene; or (ii) inhibit or interfere with activity of an expression product of a microbial antibiotic potentiator target gene. In certain embodiments of the invention the antibiotic potentiator target gene is a quinolone potentiator target gene.

[0020] The invention provides compositions comprising the inventive agents, e.g., compositions comprising a pharmaceutically acceptable carrier, excipient, vehicle, etc. In certain embodiments of the invention the composition is a sustained release formulation, e.g., for oral administration.

[0021] The invention further provides compositions comprising one or more antibiotic potentiating agents and an antibiotic whose activity is potentiated by the compound and/or whose resistance it suppresses or retards.

[0022] The invention further comprises a method of treating a subject in need thereof comprising the step of administering any of the inventive agents or compositions of the invention to the subject. Preferably an antibiotic potentiating agent is administered to a subject who also receives an antibiotic whose activity the compound potentiates. The antibiotic and the potentiating agent may be delivered together in a single composition or separately. They may be delivered by the same route of administration or different routes.

[0023] Unless otherwise indicated the present invention utilizes well known methods of molecular biology, bacteriology, cell culture, etc., as described in, for example, *Current Protocols in Molecular Biology*, and *Current Protocols in Cell Biology*, all John Wiley & Sons, N.Y., edition as of July 2002; Sambrook, Russell, and Sambrook, *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2001; Woodford, N. and Johnson, Alan, *Molecular Bacteriology: Protocols and Clinical*

Applications (Humana, 1998); and Gerhardt et al., Methods for General and Molecular Microbiology (American Society for Microbiology, 1994); each of which is incorporated herein by reference.

Brief Description of the Drawing

[0024] **Figure 1** is a schematic diagrams showing functional modules of the *E. coli* RecA protein. Amino acids numbers bracketing modules associated with particular functional activities are shown. These modules are highly conserved among bacteria. The figure is taken from Karlin and Brocchieri, 1996, and is not drawn to scale.

[0025] **Figure 2** shows results of a survival assay comparing the viability of wild type bacteria or various deletion strains following exposure to a lethal concentration of norfloxacin (right panel). Untreated control cells are shown in the left panel.

[0026] **Figure 3** is a graph comparing survival of four *E. coli* strains following exposure to a sublethal concentration of norfloxacin.

[0027] **Figure 4** presents graphs showing the effect of deletion of either *recA* or *tolC* on bacterial growth in the presence of norfloxacin (left panel) and survival following a period of exposure to norfloxacin (right panel).

[0028] **Figure 5** is a graph showing that deletion of *recA* dramatically decreases the ability of bacteria to survive exposure to norfloxacin.

[0029] **Figure 6** shows results of a colony formation assay comparing the ability of strains that carry a *recA*$^-$ allele in a quinolone resistant (grlA542) and nonresistant strain background to survive exposure to a lethal concentration of norfloxacin.

[0030] **Figure 7** is a bar graph comparing fluorescence intensity detected from wild type bacteria containing a recA:GFP reporter construct (left bars in each group) with fluorescence intensity detected from either *recA*$^+$ bacteria (middle bars in each group) or *recA*$^-$ bacteria containing the recA:GFP reporter construct (right bars in each group) in the presence of norfloxacin. The figure shows that norfloxacin causes increased transcription of the reporter gene, reflecting increased RecA activity, validating the use of this system to detect inhibitors of RecA.

[0031] **Figure 8** shows is a bar graph comparing luciferase luminescence as described in Example 7.

[0032] **Figure 9** shows the core structures of classical quinolone antibiotics (4-quinolone and 4-naphthyridine systems).

[0033] **Figure 10** shows the structural formulae and numbering systems of the 4-quinolone and 4-naphthyridine systems with the corresponding 2-quinolone and 2-naphthyridine systems and also shows a benzo[b]naphthyridone structure.

[0034] **Figure 11A** shows core structures of benzo[b]naphthyridones and some examples of this compound class. **Figure 11B** shows pyrroloquinolones, pyrazoloquinolones, and some core structures structurally related to quinolones.

[0035] **Figure 12** is Table 1, which presents the names and accession numbers of certain quinolone potentiator target genes.

[0036] **Figure 13** shows the amino acid sequence of *E. coli* RecA protein and homologs in two other bacterial species.

[0037] **Figure 14** presents chemical structures and IC_{50} data for 14 compounds that showed at least 50% inhibition of RecA ATPase activity in the luciferase assay described in Example 23.

[0038] **Figure 15** presents chemical structures of potential RecA inhibitors that are structurally related to hinokiflavone.

[0039] **Figure 16** presents chemical structures of potential RecA inhibitors that are electronically related to hinokiflavone.

[0040] **Figure 17** is a graph showing inhibition of RecA ATPase activity by hinokiflavone.

[0041] **Figure 18** is a photograph of a survival assay plate assessing the ability of various RecA inhibitors to potentiate ciprofloxacin.

[0042] **Figure 19** is a photograph of a survival assay plate assessing the ability of hinokiflavone to kill cells, and to potentiate ciprofloxacin.

[0043] **Figure 20** is a graph comparing the ability of hinokiflavone to potentiate ciprofloxacin's activity in a survival assay against a RecA+ (S3) and *recA-* (S4) strain.

[0044] **Figure 21** illustrates the method by which the docking site(s) of hinokiflavone on RecA were predicted, and also illustrates two potential sites.

[0045] **Figure 22** is a close-up view illustrating the predicted potential hinokiflavone docking sites.

[0046] **Figure 23** summarizes the results of hinokiflavone docking study predictions.

[0047] **Figure 24** highlights particular RecA residues involved in the hinokiflavone binding site.

[0048] **Figure 25** illustrates the positioning of the hinokiflavone binding site in the context of a RecA filament.

[0049] **Figure 26** represents a cross-section of a RecA filament, with the hinokiflavone binding site indicated.

[0050] **Figure 27** illustrates attractive and repulsive interactions of hinokiflavone with its binding site.

[0051] **Figure 28** illustrates the cross-species conservation of the hinokiflavone binding site.

[0052] **Figure 29** lists the names, accession numbers and descriptions of certain aminoglycoside potentiator target genes.

[0053] **Figure 30** shows results of a survival assay comparing the viability of *S. aureus* and recA defective *S. aureus* following a 4 hour-exposure of different concentrations of gentamicin.

[0054] **Figure 31** presents two graphs showing that Mefloquine (an ATPase synthase inhibitor) potentiates Gentamicin (an aminoglycoside antibiotic).

[0055] **Figure 32** lists the names, accession numbers and descriptions of certain antibiotic cyclic peptide potentiator target genes.

[0056] **Figure 33** presents a graph showing that Mefloquine (an ATPase synthase inhibitor) potentiates colistin (an antibiotic cyclic peptide).

[0057] **Figure 34** is a scheme showing overlapping target genes for quinolones (citoflaxin), aminoglycosides (gentamicin) and antibiotic cyclic peptides (colistin).

[0058] **Figure 35** is a set of 4 graphs illustrating hydroxyl radical production in *E. coli* by antibiotics. **a,c**, Log change in colony forming units per mL (CFU/mL, mean +/- standard deviation); filled squares represent a no-drug control. **b,d**, Generation of hydroxyl radicals. Representative measurements are shown and were taken 3 hours following addition of drug.

Diamonds represent time-zero baseline measurements. **a**, Survival and **b**, hydroxyl radicals generation following exposure to bactericidal antibiotics. **c**, Survival and **d**, hydroxyl radical generation following exposure to bacteriostatic drugs (see Example 39).

[0059] **Figure 36** is a set of 3 graphs showing the effect of iron chelation, hydroxyl radical quenching of iron-sulfur synthesis on the filling efficiency of bactericidal antibiotics. **a,c,e**, Log change in colony forming units per mL (CFU/mL, mean +/- standard deviation). Effect of addition of 500 μM 2,2'-dipyridyl or 150 mM thiourea to wildtype *E. coli*, and on an iron-sulfur cluster synthesis mutant, *ΔiscS*, on bactericidal drug efficacy and hydroxyl radical formation following exposure to **a**, 250 ng/mL norfloxacin (Nor), **c**, 5 μg/mL ampicillin (Amp), or **e**, 5 μg/mL kanamycin (Kan).

[0060] **Figure 37** is a set of two graphs showing SOS induction in E. coli by bactericidal antibiotics. Activation of the SOS response was monitored using an engineered sensor construct that employs the LexA repressor for control of Green Fluorescent Protein (GFP) expression. Shown are representative GFP histogram measurements taken 3 hours after addition of **a**, bactericidal antibiotics (5 μg/mL Amp, 5 μg/mL Kan, 25 ng/mL Nor). **b**, Log change in CFU/mL of *ΔrecA* E. coli following exposure to 5 μg/mL Kanamycin (open diamonds), 25 ng/mL Norfloxacin (open triangles), and 5 μg/mL Ampicillin (open circles). For comparison, CFU/mL are also shown for widltype *E. coli* exposed to 5 μg/mL Kanamycin (diamonds), 25 ng/mL Norfloxacin (triangles), and 5 μg/mL Ampicillin (circles), as well as a lower concentration of norfloxacin in these experiments to highlight the significant increase in killing in a *recA* knockout.

Definitions

[0061] *Active agent.* An "active agent" refers to an agent that either possesses antimicrobial activity, *i.e.*, inhibits growth and/or survival of a microorganism, or that potentiates an agent that possesses antimicrobial activity.

[0062] *Aliphatic*: The term "aliphatic" or "aliphatic group", as used herein, denotes a hydrocarbon moiety that may be straight-chain (*i.e.*, unbranched), branched, or cyclic (including fused, bridging, and spiro-fused polycyclic) and may be completely saturated or may contain one or more units of unsaturation, but which is not aromatic. Unless otherwise specified, aliphatic groups contain 1–20 carbon atoms. In some embodiments, aliphatic groups contain 1–10 carbon atoms. In other embodiments, aliphatic groups contain 1–8 carbon atoms. In still other embodiments, aliphatic groups contain 1–6 carbon atoms, and in yet other embodiments aliphatic groups contain 1–4 carbon atoms. Suitable aliphatic groups include, but are not limited to, linear or branched, alkyl, alkenyl, and alkynyl groups, and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[0063] *Agent.* "Agent" refers to any compound or chemical entity and includes, without limitation, small molecules, polypeptides, nucleic acids, and carbohydrates.

[0064] *Antibiotic agent.* "Antibiotic", "antibiotic agent", or "antimicrobial agent" as used herein, refers to any agent that inhibits and/or stops growth and/or proliferation of one or more species of microorganism (*e.g.*, bacteria or fungus). The antibiotic may display inhibitory activity *in vitro* (*e.g.*, when contacted with cells in cell culture), *in vivo* (e.g., when administered to a subject at risk of or suffering from an infection), or both. The terms include bactericidal and bacteriostatic agents. "Bactericidal" means that the agent kills bacteria. A bactericidal agent may inhibit or stop growth or proliferation of the bacteria before killing them. "Bacteriostatic" means that the agent substantially inhibits or stops growth or proliferation of bacteria but does not kill them.

[0065] *Antibiotic potentiator target gene.* As used herein, the term "antibiotic potentiator target gene" refers to a gene characterized in that the growth and/or viability (*i.e.*, ability to survive) of microbial cells having a deletion or functional inactivation of the gene is/are substantially reduced by exposure to an antibiotic relative to the growth and/or viability of otherwise genetically identical microbial cells not having a deletion or functional inactivation of the gene under the same conditions. The term includes any gene identified according to the gene identification methods of the invention. The exposure to the antibiotic can be either transient or continuous. The antibiotic concentration may be a sublethal concentration (*e.g.*, a non-inhibitory concentration) or a lethal concentration. The antibiotic may be any antibiotic known in the art or discovered in the future. It will be appreciated that the growth and/or viability will typically be measured with respect to a population of microbial cells and refers, for example, to the overall growth of the population or the percentage of cells that remain viable. Deletion or functional inactivation of the gene may potentiate one or more antibiotics. It is noted that the phrase "deletion or functional inactivation of a gene" is not intended to imply that "deletion" and "functional inactivation" are mutually exclusive. Deletion is one among a number of different means by which a gene can be functionally inactivated.

[0066] *Approximately.* The terms "approximately" or "about" in reference to a number generally include numbers that fall within a range of 5% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0067] *Biochemical or biological pathway.* The terms "biochemical pathway" or "biological pathway" refer to a connected series of biochemical reactions and/or molecular interactions normally occurring in a cell, or more broadly, a cellular event or process such as cell division or DNA replication. Typically, the steps of a biochemical or biological pathway occur in a coordinated fashion to produce a specific product or products or to produce a particular biochemical action, effect, or event. A biochemical or biological pathway requires the expression product of a gene (*e.g.*, the gene is essential for that pathway) if the absence of that expression product either directly or indirectly prevents the completion of one or more steps in that pathway, thereby preventing or significantly reducing the production of one or more normal products, actions, events, or effects of that pathway. Thus, an agent specifically inhibits a biological or biochemical pathway requiring the expression product of a particular gene, if the presence of the agent stops or substantially reduces the completion of the series of steps in that pathway. The agent, may, but does not necessarily, act directly on the expression product of that particular gene. A biochemical or biological pathway may involve but not require the expression products of one or more genes, in which case the absence of that expression product may either directly or indirectly reduce the extent to which one or more steps in that pathway occurs and may thereby reduce the production of one or more normal products, actions, events, or effects of that pathway. While the present invention does not specifically differentiate between use of the terms "biological pathway" and "biochemical pathway", it may be more appropriate to utilize the former where the focus is on broader cellular events or processes and to utilize the latter where the focus is on specific cellular molecules and the chemical reactions that they undergo.

[0068] *Biological or assay system.* "Biological system" or "assay system" refers to any system containing at least one biological component, *e.g.*, a biological macromolecule such as a protein or nucleic acid, suitable for performing an assay of a biological or biochemical function or activity. The term includes cell-free systems, cells, collections of cells, animals, etc.

[0069] *Conventional dose.* A "conventional dose" of an antibiotic agent means a dose that is (i) in the case of humans or animals, recommended on the package insert; (ii) in the case of humans, recommended in *Goodman and Gilman, supra*; *Katzung, supra,* and/or *The Merck Manual of Diagnosis and Therapy*, 17th ed. (1999), or the 18th ed (2006) following its publication, Mark H. Beers and Robert Berkow (eds.), Merck Publishing Group; (iii) in the case of animals, recommended in *The Merck Veterinary Manual*, 9th ed., Kahn, C.A. (ed.), *Merck Publishing Group*, 2005. It will be appreciated that a conventional dose may be modified appropriately for an individual subject taking into account, for example, factors such as the subject's age, diet, renal and/or hepatic function, other medications, other diseases or conditions (*i.e.*, diseases or conditions other than the infection for which an antibiotic agent is administered), past experience with the antibiotic agent, etc.

[0070] *Docking.* "Docking" is used herein consistently with its use in the art to refer to the application of any of a variety of computational methods to predict or identify a preferred structure of a complex formed by a receptor and ligand. Docking typically involves computational exploration of the possible binding modes of a ligand to a receptor and identification of low energy binding modes. The receptor can be, *e.g.*, a macromolecule such as a polypeptide, protein, or nucleic acid. The ligand can be, *e.g.*, a small molecule.

[0071] *Effective amount.* An "effective amount" of an agent refers to the amount of the agent sufficient to elicit a desired biological response. As will be appreciated by those of ordinary skill in this art, the absolute amount of a particular agent that is effective may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the target tissue, *etc.* Those of ordinary skill in the art will further understand that an "effective amount" may be administered in a single dose, or may be achieved by administration of multiple doses. For example, an effective amount may be an amount sufficient to achieve one or more of the following: (i) inhibit microbial growth in culture or *in vivo*; (ii) reduce the severity of or prevent one or more symptoms or signs of an infection; (iii) significantly reduce the risk of recurrence of an infection; (iv) significantly reduce the risk of a clinically significant infection in a subject who has been exposed to an infectious agent, etc.

[0072] Comparably, an effective amount of a potentiating agent may be an amount sufficient to achieve the same level of antibiotic activity with a particular antibiotic agent as is achieved when that antibiotic agent is administered at its conventional dose, in circumstances where the antibiotic agent is administered at a reduced dose as compared with its conventional dose.

[0073] For example, an effective amount of a RecA inhibitor according to the present invention may be, for example, (i) an amount sufficient to act as an antibiotic agent; (ii) an amount sufficient to inhibit one ore more activities of RecA (or a relevant homolog); (iii) an amount sufficient to potentiate activity of one ore more antibiotic agents (*e.g.*, with which the RecA inhibitor is administered in combination); and/or (iv) an amount sufficient to reduce or retard the incidence of resistance developed to another antibiotic agent (*e.g.*, with which the RecA is administered in combination) .

[0074] *Essential gene.* An "essential gene" is one whose deletion or functional inactivation renders a microorganism lacking the gene incapable of growth or proliferation and/or causes the cell to die. It will be appreciated that whether or not a particular gene is essential depends at least in part on the environmental conditions to which the cell is subjected.

[0075] *Gene.* The term "gene" refers to a nucleic acid comprising a nucleotide sequence that encodes a polypeptide or a biologically active ribonucleic acid (RNA) such as a tRNA. The nucleic acid can further include regulatory elements (*e.g.*, expression control sequences such as promoters, enhancers, etc.) and/or introns. A gene that encodes a polypeptide contains one or more coding portions (open reading frames), by which is meant a series of nucleotide triplets coding for amino acids uninterrupted by termination codons, wherein the sequence can be translated into a polypeptide using the codon usage information appropriate for a particular cell or multicellular organism.

[0076] *Gene product or expression product.* A "gene product" or "expression product" of a gene is an RNA transcribed from the gene (*e.g.*, either pre- or post-processing) or a polypeptide encoded by an RNA transcribed from the gene (*e.g.*, either pre- or post-modification).

[0077] *Growth* and *Proliferation.* "Growth" refers to an increase in microbial biomass. "Proliferation" refers to an increase in microbial number. Since bacterial proliferation, rather than mere increase in cell mass without cell division, is usually of primary concern, and since under most circumstances of interest herein proliferation is accompanied by an increase in microbial biomass, the term "growth" is generally understood to mean "proliferation", and the two terms are used interchangeably herein although it is recognized that different assays may measure either or both of these parameters. For example, optical density reflects biomass and does not specifically reflect cell number, whereas an assay based on detecting colonies formed from individual cells reflects cell number rather than biomass.

[0078] *Heteroatom*: The term "heteroatom" means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon. This includes any oxidized form of nitrogen, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen, or; a substitutable nitrogen of a heterocyclic ring including =N– as in 3,4-dihydro-2*H*-pyrrolyl, –NH– as in pyrrolidinyl, or =N($R^{\dagger}$)– as in N-substituted pyrrolidinyl.

[0079] *High throughput screening*. "High throughput screening" as used herein refers to an assay that allows for multiple candidate agents or samples to be screened substantially simultaneously. Such assays typically entail the use of microtiter (microwell) plates (*e.g.*, plates having 96, 384, or 1596 wells) which are particularly convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. Such assays may also advantageously minimize the number of steps such as washing cells, removing culture medium, and/or pipetting reagents.

[0080] *Hypersensitivity*. "Hypersensitive", "hypersensitivity", "hypersusceptible", "hyper-susceptibility" and like terms refer to a cell that exhibits increased sensitivity to inhibition of growth and/or survival by an antibiotic as a consequence of a gene being underexpressed or as a consequence of a gene product being inhibited or interfered with by an agent (*i.e.*, a potentiating agent), relative to the sensitivity that the cell would exhibit to that antibiotic if the gene was not underexpressed or the gene product not inhibited or interfered with. Typically the minimum inhibitory concentration (MIC) of that antibiotic will have been reduced in a cell that is hypersensitive to the antibiotic.

[0081] *In combination*. "In combination" as used herein with respect to administration of first and second agents is administration performed such that (i) a dose of the second agent is administered before more than 90% of the most recently administered dose of the first agent has been metabolized to an inactive form or excreted from the body; or (ii) doses of the first and second agents are administered within 48 hours of each other, or (iii) the agents are administered during overlapping time periods (*e.g.*, by continuous or intermittent infusion); or (iv) any combination of the foregoing. The agents may, but need not, be administered together as components of a single composition. The agents may be administered individually at substantially the same time (by which is meant within less than 10 minutes of one another). The agents may be administered individually within a short time of one another (by which is meant less than 1 hour apart). The agents may, but need not, be administered by the same route of administration. When administered in combination with a second agent, the effective concentration of a first agent needed to elicit a particular biological response may be less than the effective concentration of the first agent when administered in the absence of the second agent, thereby allowing a reduction in the dose of the first agent relative to the dose that would be needed if the first agent was administered in the absence of the second agent. The effects of multiple agents may, but need not be, additive or synergistic. The agents may be administered multiple times. In some embodiments of the invention, a lower amount of first agent (*e.g.*, antibiotic agent) is required in the presence of the second agent (*e.g.*, inventive RecA inhibitor). The effects of multiple agents may, but need not be, additive or synergistic. One or more of the agents may be administered multiple times.

[0082] *Identity.* "Identity" refers to the extent to which the sequence of two or more nucleic acids or polypeptides is the same. The percent identity between a sequence of interest and a second sequence over a window of evaluation, *e.g.*, over the length of the sequence of interest, may be computed by aligning the sequences, determining the number of residues (nucleotides or amino acids) within the window of evaluation that are opposite an identical residue allowing the introduction of gaps to maximize identity, dividing by the total number of residues of the sequence of interest or the second sequence (whichever is greater) that fall within the window, and multiplying by 100. When computing the number of identical residues needed to achieve a particular percent identity, fractions are to be rounded to the nearest whole number. Percent identity can be calculated with the use of a variety of computer programs known in the art. For example, computer programs such as BLAST2, BLASTN, BLASTP, Gapped BLAST, etc., generate alignments and provide percent identity between a sequence of interest and sequences in any of a variety of public databases. The algorithm of Karlin and Altschul (Karlin and Altschul, Proc. Natl. Acad. Sci. USA, 1990, 87: 22264-2268) modified as in Karlin and Altschul, Proc. Natl. Acad. Sci. USA, 1993, 90: 5873-5877 is incorporated into the NBLAST and XBLAST programs of Altschul et al. (Altschul *et al.*, J. Mol. Biol., 1990, 215: 403-410). To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul *et al.* (Altschu et al., Nucleic Acids Res., 1997, 25: 3389-3402). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used. A PAM250 or BLOSUM62 matrix may be used. See the web page having URL www.ncbi.nlm.nih.gov at the National Center for Biotechnology Information website for these programs. In a specific embodiment, percent identity of a sequence of interest and a second sequence is calculated using BLAST2 with default parameters.

[0083] *Isolated.* The term "isolated" means 1) separated from at least some of the macromolecular components with which it is usually associated in nature; 2) prepared or purified by a process that involves the hand of man; and/or 3) not occurring in nature. For example, an "isolated bacterial cell" is a cell that is not located in or on a multicellular organism, *e.g.*, the cell is maintained in culture. An "isolated polypeptide" is one that is not present in a cell or at the surface of a cell in which the polypeptide was synthesized. The isolated polypeptide may be present in a cell-free solution.

[0084] *Microbe.* "Microbe", "microbial", and like terms, as used herein, refers to microscopic organisms, *e.g.*, bacteria or fungi, particular bacteria. For example, when used in reference to quinolone antibiotics, "microbe" and like terms typically refer to bacteria, although it can encompass any microorganism against which quinolone antibiotics display inhibitory activity.

[0085] *Microbial infection.* The term "microbial infection" refers to the invasion of the host organism, whether the organism is a vertebrate, invertebrate, fish, plant, bird, or mammal, by pathogenic microbes, *e.g.*, bacteria, fungi, and protists. . This includes the excessive growth of microbes that are normally present in or on the body of a mammal or other organism. More generally, a microbial infection can be any situation in which the presence of a microbial population(s) is damaging to a host organism. Thus, an organism is "suffering" from a microbial infection when excessive numbers of a microbial population are present in or on the organism's body, or when the effects of the presence of a microbial population(s) is damaging the cells or other tissue of an organism. The agents and compositions of certain embodiments of the invention are also useful in treating microbial growth or contamination of cell cultures or other media, or inanimate surfaces or objects, and nothing herein should limit the invention to treatment of higher organisms, except when explicitly so specified in the claims.

[0086] *Minimal inhibitory concentration (MIC):* The terms "minimal inhibitory concentration" (MIC) and "minimal bactericidal concentration" (MBC) are used herein consistently with their use in the art, *i.e.*, to indicate the concentration of an agent that will inhibit bacterial proliferation (growth) (MIC) or kill bacteria (MBC). MIC values may be for example, the concentration of agent that inhibits visible growth or may be expressed as MIC_{50}, MIC_{90} or MIC_{99} values *i.e.*, the concentration of an agent that reduces bacterial proliferation to 50% or less, 10% or less, or 1% or less, respectively, of the control value that would occur in the absence of the agent. As is well known in the art, MIC and MBC can be measured by a variety of methods, including automated and non-automated methods. Suitable methods are described in publications of the Clinical Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS), as set forth in NCCLS: Performance Standards documents referenced below.

[0087] *Modulation.* To "modulate" means to change or alter, *e.g.*, to cause an increase or decrease. Thus an agent is said modulate an amount or activity if it causes the amount or activity to change or alter, *e.g.*, to increase or decrease. An agent that inhibits or activates expression of a gene or activity of a gene product is said to modulate expression of the gene or modulate activity of the gene product, respectively.

[0088] *Naturally-occurring.* As used herein, a "naturally occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural polypeptide). A "naturally occurring" polypeptide is a polypeptide having an amino acid sequence that occurs in nature.

[0089] *Operably linked or associated.* "Operably linked" or "operably associated" refers to a functional relationship between two nucleic acids, wherein the expression, activity, localization, etc., of one of the sequences is controlled by, directed by, regulated by, modulated by, etc., the other nucleic acid. The two nucleic acids are said to be operably linked or operably associated. "Operably linked" or "operably associated" can also refers to a relationship between two polypeptides wherein the expression of one of the polypeptides is controlled by, directed by, regulated by, modulated by, etc., the other polypeptide. The two polypeptides are said to be operably linked or operably associated. For example, transcription of a nucleic acid is directed by an operably linked promoter; post-transcriptional processing of a nucleic acid is directed by an operably linked processing sequence; translation of a nucleic acid is directed by an operably linked translational regulatory sequence such as a translation initiation sequence; transport, stability, or localization of a nucleic acid or polypeptide is directed by an operably linked transport or localization sequence such as a secretion signal sequence; and post-translational processing of a polypeptide is directed by an operably linked processing sequence. Preferably a first nucleic acid sequence that is operably linked to a second nucleic acid sequence, or a first polypeptide that is operatively linked to a second polypeptide, is covalently linked, either directly or indirectly, to such a sequence, although any effective three-dimensional association is acceptable. One of ordinary skill in the art will appreciate that multiple nucleic acids, or multiple polypeptides, may be operably linked or associated with one another.

[0090] *Overexpression.* "Overexpressed" or "overexpression" means expression of a gene at levels above the wild type levels observed for the gene in organisms of that type under the same conditions, *i.e.*, expression of the gene is increased relative to wild type levels. For example, the value of the steady state or average rate of synthesis or absolute amount of an expression product of the gene (*e.g.*, a polypeptide) may be at greater than the wild type value by at least a factor of 1.5, at least a factor of 2, at least a factor of 5, at least a factor of 10, etc.

[0091] *Pharmaceutically acceptable derivative*: According to the present invention, a pharmaceutically acceptable derivative of a particular chemical compound includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or any other adduct or derivative which upon administration to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof. Thus, pharmaceutically acceptable derivatives can include salts, prodrugs, and/or metabolites of relevant compounds. The phrase "pharmaceutically acceptable derivative" may also encompass quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersable products may be obtained by such quaternization.

[0092] *Pharmaceutically acceptable salt*: As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and which are commensurate with a reasonable benefit/risk ratio. A "pharmaceutically acceptable salt" means any non-toxic salt or salt of an ester of a compound of this invention that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an inhibitorally active metabolite or residue thereof. As used herein, the term "inhibitorally active metabolite or residue thereof" means that a metabolite or residue thereof acts as a RecA inhibitor.

[0093] A wide variety of appropriate pharmaceutically acceptable salts are well known in the art. For example, S.M. Berge *et al.*, describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 66:1, 1977, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases.

[0094] Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like.

[0095] Examples of pharmaceutically acceptable salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}\text{ alkyl})_4$ salts.

[0096] Representative pharmaceutically acceptable alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like.

[0097] Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations, for example formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

[0098] *Physiologically acceptable carrier or excipient*: As used herein, the term "physiologically acceptable carrier or excipient" refers to a carrier medium or excipient which does not interfere with the effectiveness of the biological activity of the active ingredients and which is not excessively toxic to the host at the concentrations at which it is administered. The term includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic agents, absorption delaying agents, and the like. The use of such media and agents for the formulation of pharmaceutically active substances is well-known in the art (see, for example, "*Remington's Pharmaceutical Sciences*", E.W. Martin, 18th Ed., 1990, Mack Publishing Co.: Easton, PA, which is incorporated herein by reference in its entirety).

[0099] *Polynucleotide.* The terms "polynucleotide" and "nucleic acid" are used interchangeably herein to refer to a polymer of nucleotides, *e.g.*, DNA or RNA. The polymer may comprise or natural nucleosides found in DNA or RNA, other nucleosides or nucleoside analogs, nucleosides containing chemically modified bases and/or biologically modified bases (*e.g.*, methylated bases), modified sugars, modified backbone linkages, etc. The nucleic acid molecule can be single-stranded or double-stranded or can contain single-stranded regions and double-stranded regions. The term "polynucleotide sequence" or "nucleic acid sequence" as used herein can refer to the nucleic acid material itself and/or to the sequence information (*i.e.* the succession of letters chosen among the five base letters A, G, C, T, or U) that biochemically characterizes a specific nucleic acid, *e.g.*, a DNA or RNA molecule. A nucleic acid sequence is presented in the 5' to 3' direction herein unless otherwise indicated.

[00100] *Polypeptide.* "Polypeptide", as used herein, refers to a chain of amino acids connected by peptide bonds. A protein is a molecule composed of one or more polypeptides. Each polypeptide in a protein may be referred to as a "subunit" of the protein. A peptide is a relatively short polypeptide, typically between about 2 and 60 amino acids in length. The terms "protein", "polypeptide", and "peptide" may be used interchangeably herein unless indicated otherwise. Polypeptides as used herein typically contain amino acids such as those that are naturally found in proteins. However, amino acids that are not naturally found in proteins (*e.g.*, amino acids that do not occur in nature or that do occur in nature but are not naturally found in proteins and that can be incorporated into a polypeptide chain), and/or amino acid analogs can also or alternatively be used in polypeptides that are employed in the screening assays of the invention. One or more of the amino acids in a polypeptide may be modified, for example, by the addition of one or more chemical entities such as a carbohydrate group, a phosphate group, a lipid group, etc. Specific examples include a farnesyl group, an isofarnesyl group, a fatty acid group, a glycosyl group, an acetyl group, etc. Polypeptides may contain a linker for conjugation, functionalization, or other modification, etc. The term "polypeptide sequence" or "amino acid sequence" as used herein can refer to the polypeptide material itself and is not restricted to the sequence information (*i.e.* the succession of letters or three letter codes chosen among the letters and codes used as abbreviations for amino acid names) that biochemically characterizes a polypeptide. A polypeptide sequence presented herein is presented in an N-terminal to C-terminal direction unless otherwise indicated.

[00101] *Potentiate*: The term "potentiate", as used herein, means to enhance or increase at least one biological effect or activity of a biologically and/or pharmacologically active agent so that either (i) a given concentration or amount of the agent results in a greater biological effect or activity when the agent is potentiated than the biological effect or activity that would result from the same concentration or amount of the agent when not potentiated; or (ii) a lower concentration or amount of the agent is required to achieve a particular biological effect or activity when the agent is potentiated than when the agent is not potentiated; or (iii) both (i) and (ii). The biological effect or activity may be, for example, the ability to catalyze or inhibit one or more chemical reactions, the ability to activate or inhibit a biological or biochemical pathway, the ability to reduce or inhibit microbial proliferation, the ability to kill a microorganism, etc. An agent whose presence potentiates another agent may be referred to as a "potentiating agent". A potentiating agent may show biological activity by itself, or may exhibit biological activity only when used in combination with a biologically and/or pharmacologically active agent.

[00102] *Protist*. The term "protist" refers to any member of a diverse group of organisms, comprising those eukaryotes that are not animals, plants or fungi. Protists can be unicellular or multicellular. Protists are group in three subcategories: animal-like protists, fungus-like protists, and plant-like protists.

[00103] *Purified*. "Purified", as used herein, means that an entity or substance is separated from one or more other entities or substances with which it was previously found before being purified. An entity or substance may be partially purified, substantially purified, or pure. A substance or entity such as a nucleic acid or polypeptide is considered pure when it is removed from substantially all other compounds or entities other than a solvent and any ions contained in the solvent, *i.e.*, it constitutes at least about 90%, more preferably at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater than 99% of the dry weight of the composition. A partially or substantially purified compound or entity such as a nucleic acid or polypeptide may be removed from at least 50%, at least 60%, at least 70%, or at least 80% of the material with which it is naturally found, e.g., cellular material such as cellular proteins and/or nucleic acids. In certain embodiments of the

invention a purified nucleic acid, polypeptide, or protein constitutes at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99% or even more, by dry weight, of the total nucleic acid, polypeptide, or protein, respectively, in a composition. Methods for purifying nucleic acids, polypeptides, and proteins and for assessing purity are known in the art and include spectophotometric methods, chromatrographic methods, immunological methods, electrophoretic methods, etc.

[00104] *Sensitive/susceptible* and *resistant*. A microorganism is "sensitive" or "susceptible" to an agent if the agent inhibits proliferation of the microorganism and/or kills the microorganism when contacted with the agent at a particular concentration. Sensitivity may be assessed using any of a variety of methods known in the art. A microorganism that is not "sensitive" is considered "resistant", *i.e.*, the microorganism can survive and proliferate in the presence of the agent. Methods for assessing sensitivity typically involve determining the MIC by methods such as the broth microdilution method, agar dilution, and the agar disk diffusion method. The MIC may then be compared with a predefined "breakpoint", wherein a MIC greater than the breakpoint indicates that the microorganism is resistant to the agent and a MIC equal to or below the breakpoint indicates that the microorganism is sensitive to the agent. Sensitivity and/or resistance may be assessed according to the guidelines and methods established by the Clinical Laboratory Standards Institute (CLSI), formerly the National Commmittee for Clinical Laboratory Standards (NCCLS), as set forth in NCCLS: Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement. NCCLS document M100-S14. Wayne, PA: NCCLS 2004; NCCLS: Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eighth Edition. NCCLS document M2-A8. Wayne, PA : NCCLS 2003; or NCCLS: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard-Sixth Edition. NCCLS document M7-A6. Wayne, PA : NCCLS 2003. "Intrinsic resistance" means that a bacterial species is inherently resistant to the effects of an antibacterial agent. "Acquired resistance" means that a bacterial species, subtype, or strain has acquired a mechanism of resistance since the introduction of the antibacterial agent into use. Resistance may, for example, be acquired by mutation of a target gene, by overexpression of an efflux pump, etc. A subpopulation of a bacterial species that has acquired resistance may be considered a distinct strain of that species.

[00105] *RecA Inhibitor*. According to the present invention, an agent is a RecA inhibitor if one or more RecA activities is reduced in the agent's presence as compared with its

absence, or if the level or amount of RecA protein or gene product is reduced in the agent's presence as compared with its absence. In certain embodiments, RecA inhibitors act directly on RecA in that they physically interact with RecA. In other embodiments, inhibitors act indirectly on RecA.

[00106] *Small Molecule.* In general, a small molecule is understood in the art to be an organic molecule that is less than about kilodaltons (KDa) in size. In some embodiments, the small molecule is less than about 3 KDa, 2 KDa, or 1 KDa. In some embodiments, the small molecule is less than about 800 daltons (Da), 600 Da, 500 Da, 400 Da, 300 Da, 200 Da, or 100 Da. In some embodiments, small molecules are non-polymeric. In some embodiments, small molecules are not amino acids. In some embodiments, small molecules are not nucleotides. In some embodiments, small molecules are not saccharides.

[00107] *Specific binding.* "Specific binding" generally refers to a physical association between a target polypeptide (or, more generally, a target molecule) and a binding molecule such as an antibody or ligand. The association is typically dependent upon the presence of a particular structural feature of the target such as an antigenic determinant or epitope recognized by the binding molecule. For example, if an antibody is specific for epitope A, the presence of a polypeptide containing epitope A or the presence of free unlabeled A in a reaction containing both free labeled A and the binding molecule that binds thereto, will reduce the amount of labeled A that binds to the binding molecule. It is to be understood that specificity need not be absolute but generally refers to the context in which the binding occurs. For example, it is well known in the art that numerous antibodies cross-react with other epitopes in addition to those present in the target molecule. Such cross-reactivity may be acceptable depending upon the application for which the antibody is to be used. One of ordinary skill in the art will be able to select antibodies or ligands having a sufficient degree of specificity to perform appropriately in any given application (*e.g.*, for detection or inhibition of a target molecule, for therapeutic purposes, etc). It is also to be understood that specificity may be evaluated in the context of additional factors such as the affinity of the binding molecule for the target versus the affinity of the binding molecule for other targets, *e.g.*, competitors. If a binding molecule exhibits a high affinity for a target molecule that it is desired to detect and low affinity for nontarget molecules, the antibody will likely be an acceptable reagent. Once the specificity of a binding molecule is established in one or more contexts, it may be employed in other, preferably similar, contexts without necessarily re-evaluating its specificity. Binding of two or more entities (*e.g.*, a receptor and a ligand, an antibody and an antigen, etc.) may be considered specific if the equilibrium dissociation constant (Kd) of a complex formed by the two or more entities is 10^{-4} M or less, preferably 10^{-5} M or less, *e.g.*, 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less, or 10^{-10} M or less under the conditions tested.

[00108] *Strain.* A "strain" is a genetic variant or subtype of a type or species or microorganism, *e.g.*, an isolate of a microorganism that possesses the major properties that define the species or type but differs from many or most other members of the species or type in one or more other properties. The term "strain" can refer to a bacterium that harbors a particular episome or contains a particular mutation in a gene that is not found in many other subtypes or strains of the species.

[00109] *Subject.* "Subject", as used herein, refers to an individual to whom an agent is to be delivered, *e.g.*, for experimental, diagnostic, and/or therapeutic purposes. Subjects of interest herein include animals, particularly agriculturally significant animals or companion animals (*e.g.*, cows, sheep, goats, horses, swine, dogs, cats, rabbits, birds, fish, etc.), laboratory animals (*e.g.*, mice, rats) primates, or humans.

[00110] *Sublethal.* A "sublethal" concentration of an antibiotic refers to a concentration that is less than the MIC of the antibiotic. In certain embodiments of the invention a sublethal concentration is not sufficient to significantly reduce the growth rate (proliferation) of a microbial cell, *e.g.*, the growth rate is reduced by less than 20%, preferably less than 10%. Such a concentration is referred to herein as a "non-inhibiting concentration". A "lethal" concentration of an antibiotic is one that is equal to or greater than the MIC and would ultimately result in microbial death and complete or essentially complete sterilization of a culture medium containing the microbe if continued indefinitely assuming that no resistant strains arise during the incubation period.

[00111] *Substituted.* As described herein, compounds of the invention may contain "optionally substituted" moieties. In general, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are

preferably those that result in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[00112] Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group are independently halogen; $-(CH_2)_{0-4}R^{\circ}$; $-(CH_2)_{0-4}OR^{\circ}$; $-O-(CH_2)_{0-4}C(O)OR^{\circ}$; $-(CH_2)_{0-4}CH(OR^{\circ})_2$; $-(CH_2)_{0-4}SR^{\circ}$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; $-CH{=}CHPh$, which may be substituted with R°; $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^{\circ})_2$; $-(CH_2)_{0-4}N(R^{\circ})C(O)R^{\circ}$; $-N(R^{\circ})C(S)R^{\circ}$; $-(CH_2)_{0-4}N(R^{\circ})C(O)NR^{\circ}_2$; $-N(R^{\circ})C(S)NR^{\circ}_2$; $-(CH_2)_{0-4}N(R^{\circ})C(O)OR^{\circ}$; $-N(R^{\circ})N(R^{\circ})C(O)R^{\circ}$; $-N(R^{\circ})N(R^{\circ})C(O)NR^{\circ}_2$; $-N(R^{\circ})N(R^{\circ})C(O)OR^{\circ}$; $-(CH_2)_{0-4}C(O)R^{\circ}$; $-C(S)R^{\circ}$; $-(CH_2)_{0-4}C(O)OR^{\circ}$; $-(CH_2)_{0-4}C(O)SR^{\circ}$; $-(CH_2)_{0-4}C(O)OSiR^{\circ}_3$; $-(CH_2)_{0-4}OC(O)R^{\circ}$; $-OC(O)(CH_2)_{0-4}SR-$, $SC(S)SR^{\circ}$; $-(CH_2)_{0-4}SC(O)R^{\circ}$; $-(CH_2)_{0-4}C(O)NR^{\circ}_2$; $-C(S)NR^{\circ}_2$; $-C(S)SR^{\circ}$; $-SC(S)SR^{\circ}$, $-(CH_2)_{0-4}OC(O)NR^{\circ}_2$; $-C(O)N(OR^{\circ})R^{\circ}$; $-C(O)C(O)R^{\circ}$; $-C(O)CH_2C(O)R^{\circ}$; $-C(NOR^{\circ})R^{\circ}$; $-(CH_2)_{0-4}SSR^{\circ}$; $-(CH_2)_{0-4}S(O)_2R^{\circ}$; $-(CH_2)_{0-4}S(O)_2OR^{\circ}$; $-(CH_2)_{0-4}OS(O)_2R^{\circ}$; $-S(O)_2NR^{\circ}_2$; $-(CH_2)_{0-4}S(O)R^{\circ}$; $-N(R^{\circ})S(O)_2NR^{\circ}_2$; $-N(R^{\circ})S(O)_2R^{\circ}$; $-N(OR^{\circ})R^{\circ}$; $-C(NH)NR^{\circ}_2$; $-P(O)_2R^{\circ}$; $-P(O)R^{\circ}_2$; $-OP(O)R^{\circ}_2$; $-OP(O)(OR^{\circ})_2$; SiR°_3; $-(C_{1-4}$ straight or branched alkylene$)O-N(R^{\circ})_2$; or $-(C_{1-4}$ straight or branched alkylene$)C(O)O-N(R^{\circ})_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3–12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[00113] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(CH_2)_{0-2}R^{\bullet}$, $-(haloR^{\bullet})$, $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^{\bullet}$, $-(CH_2)_{0-2}CH(OR^{\bullet})_2$; $-O(haloR^{\bullet})$, $-CN$, $-N_3$, $-(CH_2)_{0-2}C(O)R^{\bullet}$, $-(CH_2)_{0-2}C(O)OH$, $-(CH_2)_{0-2}C(O)OR^{\bullet}$, $-(CH_2)_{0-2}SR^{\bullet}$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^{\bullet}$, $-(CH_2)_{0-2}NR^{\bullet}_2$, $-NO_2$, $-SiR^{\bullet}_3$, $-OSiR^{\bullet}_3$, $-C(O)SR^{\bullet}$, $-(C_{1-4}$ straight or branched alkylene$)C(O)OR^{\bullet}$, or $-SSR^{\bullet}$ wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

[00114] Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, $=NNR^*_2$, $=NNHC(O)R^*$, $=NNHC(O)OR^*$, $=NNHS(O)_2R^*$, $=NR^*$, $=NOR^*$, $-O(C(R^*_2))_{2-3}O-$, or $-S(C(R^*_2))_{2-3}S-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6-membered saturated, partially unsaturated, or aryl ring having 0_4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: $-O(CR^*_2)_{2-3}O-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00115] Suitable substituents on the aliphatic group of R^* include halogen, $-R^\bullet$, $-(haloR^\bullet)$, -OH, $-OR^\bullet$, $-O(haloR^\bullet)$, -CN, -C(O)OH, $-C(O)OR^\bullet$, $-NH_2$, $-NHR^\bullet$, $-NR^\bullet_2$, or $-NO_2$, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00116] Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include $-R^\dagger$, $-NR^\dagger_2$, $-C(O)R^\dagger$, $-C(O)OR^\dagger$, $-C(O)C(O)R^\dagger$, $-C(O)CH_2C(O)R^\dagger$, $-S(O)_2R^\dagger$, $-S(O)_2NR^\dagger_2$, $-C(S)NR^\dagger_2$, $-C(NH)NR^\dagger_2$, or $-N(R^\dagger)S(O)_2R^\dagger$; wherein each $R^\dagger$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted -OPh, or an unsubstituted 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^\dagger$, taken together with their intervening atom(s) form an unsubstituted 3–12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00117] Suitable substituents on the aliphatic group of $R^{\dagger}$ are independently halogen, $-R^{\bullet}$, $-(haloR^{\bullet})$, -OH, $-OR^{\bullet}$, $-O(haloR^{\bullet})$, -CN, -C(O)OH, $-C(O)OR^{\bullet}$, $-NH_2$, $-NHR^{\bullet}$, $-NR^{\bullet}_2$, or $-NO_2$, wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00118] *Survival*. The term "survival", as used herein, refers to an ability of microbial cells to grow in the presence of one or more antibiotic agent(s) present above the relevant minimum inhibitory concentration. In some embodiments, survival is assessed at a concentration that is at or above a multiple of MIC (*e.g.*, 2x, 4x, 5x, 6x, 8x, 10x, etc)

[00119] Target gene. The term "target gene" is used as understood in the art to refer to any gene, wherein modulating expression of the gene or modulating activity of an expression product of the gene would be expected to or does achieve a biological effect. The term "target" is used to refer to either a target gene or an expression product of a target gene, *e.g.*, an RNA transcribed from the gene or more typically, a polypeptide encoded by the gene, or a protein of which the polypeptide is a subunit. The terms "target" and "target gene" are used for purposes of convenience and are not intended to be limiting. By way of example, any polypeptide or other molecule or portion thereof whose synthesis by a microbial cell is needed in order for an antibiotic agent to inhibit growth and/or survival of the cell may be a target molecule, and any gene that encodes such a polypeptide may be a target gene.

[00120] *Toxicity*. "Toxicity" refers to any adverse and/or undesired effect of a composition on the metabolism or functioning of a cell, tissue, organ or body part, or subject. The amount of toxicity associated with a composition may vary with several conditions including, but not limited to, the amount of composition present, the components present in the composition, the formulation of the composition, the environmental conditions and physiological state of the cell, tissue, organ or body part, or subject, etc.

[00121] *Treatment*. "Treating" or "treatment of" as used herein, refers to providing any type of medical or surgical management to a subject. Treating can include, but is not limited to, administering a pharmaceutical composition to a subject. Treating is typically undertaken in an effort to alter the course of a disease, disorder, or undesirable condition in a manner beneficial to the subject. The effect of treating can generally include reversing, alleviating, reducing, inhibiting the progression of, and/or reducing the likelihood of

occurrence of the disease, disorder, or condition to which such term applies, or one or more symptoms or manifestations of such disease, disorder or condition. A composition of this invention can be administered to a subject who has developed an infection or is at increased risk of developing an infection relative to a member of the general population. A composition of this invention can be administered prophylactically, *i.e.*, before development of any symptom or manifestation of a condition. Typically in this case the subject will be at risk of developing the condition. The composition can be administered prior to exposure of the subject to an infectious agent or prior to the occurrence of a pathogenic event.

[00122] *Underexpression.* "Underexpressed" or "underexpression" means expression of a gene at levels below the wild type levels observed for the gene in organisms of that type under the same conditions, *i.e.*, expression of the gene is decreased relative to wild type levels. For example, the value of the steady state or average rate of synthesis or absolute amount of an expression product of the gene (*e.g.*, a polypeptide encoded by the gene), may be between 0% and 95% of the wild type value.

[00123] *Unit Dosage Form.* A "unit dosage form", as that term is used herein, refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active agent(s) calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier

[00124] *Unsaturated.* The term "unsaturated", as used herein, means that a moiety has one or more units of unsaturation.

[00125] *Vector.* "Vector" is used herein to refer to a nucleic acid or a virus or portion thereof (*e.g.*, a viral capsid) capable of mediating entry of, *e.g.*, transferring, transporting, etc., a nucleic acid molecule into a cell. Where the vector is a nucleic acid, the nucleic acid molecule to be transferred is generally linked to, *e.g.*, inserted into, the vector nucleic acid molecule. A nucleic acid vector may include sequences that direct autonomous replication within suitable host cells (*e.g.*, an origin of replication), or may include sequences sufficient to allow integration of part of all of the nucleic acid into host cell DNA. Useful nucleic acid vectors include, for example, DNA or RNA plasmids, cosmids, and naturally occurring or modified viral genomes or portions thereof or nucleic acids that can be packaged into viral capsids. An "expression vector" is a vector suitable for expressing a nucleic acid or polypeptide in a host cell. The expression vector typically includes regulatory elements (*e.g.*, promoters, enhancers, and/or other expression signals and, optionally, additional

regulatory elements such as termination signals sufficient to direct transcription of a nucleic acid segment to which they are operably linked. The expression vector may also comprise operably linked sequences required for proper translation of the nucleic acid segment. The nucleic acid segment may, but need not be, a protein coding sequence. The nucleic acid segment may be chimeric, meaning that it includes more than one sequence of distinct origin that are joined together by recombinant DNA techniques, resulting in a nucleotide sequence that does not occur naturally. The term "expression vector" can refer to a vector either before or after insertion of the operably linked nucleic acid segment that is to be transcribed. Certain expression vectors allow the shuttling of DNA between hosts such as bacteria-yeast, or bacteria-animal cells, or bacteria-fungal cells, or bacteria-invertebrate cells. A typical expression vector will contain an origin of replication for autonomous replication in host cells, one or more selectable markers, one or more (typically several) useful restriction enzyme sites, frequently a potential for high copy number, and one or more promoters.

Detailed Description of Certain Embodiments of the Invention

I. Overview

[00126] Various aspects of the present invention are directed towards (i) methods of screening to identify target genes for the development of new agents to be used to combat bacterial infection, (ii) target genes identified according to the methods and the expression products of these target genes, (iii) methods, reagents, and assay systems for the identification of agents that modulate expression and/or activity of the targets, (iv) agents that modulate, *e.g.*, inhibit, expression and/or activity of the targets, and (v) methods of treating a subject in need thereof using the agents and compositions containing the agents. An agent is said to potentiate an antibiotic if, for example, (i) the concentration of antibiotic needed to achieve a given effect is lowered if the agent and the antibiotic are used in combination; (ii) the spectrum of microorganisms whose growth or survival can be inhibited is expanded if the agent and the antibiotic are used in combination; (iii) a microorganism that would otherwise be resistant to the antibiotic is sensitive to it (or to a particular concentration of it) if the agent and the antibiotic are used in combination.

[00127] The antibiotic potentiating agent may work by any of a variety of different mechanisms. It may or may not affect the same molecular target or biological or biochemical pathway as the antibiotic(s) whose activity it potentiates. The potentiating agent may inhibit or alter metabolism of the antibiotic by the microorganism. For example, the potentiating agent may be an inhibitor (*e.g.*, a competitive or noncompetitive inhibitor) of a microbial enzyme that degrades the antibiotic. The agent and the antibiotic may exhibit a "synthetic lethal" effect, *i.e.*, the combination of the two is lethal at concentrations at which neither is lethal by itself. Preferably the compound is substantially nontoxic to eukaryotic cells, *e.g.*, mammalian cells, over a range of concentrations, including concentrations at which it effectively potentiates the activity of an antibiotic. Typically the potentiating agent potentiates the antibiotic by a mechanism that does not substantially involve alterating the absorption, metabolism, distribution, or excretion (ADME) of the antibiotic by the subject to which it is administered, *i.e.*, the potentiating agent would potentiate the antibiotic regardless of any alterations in ADME that the agent may cause.

[00128] In certain embodiments of the invention a combination of the antibiotic and the potentiating agent is therapeutically effective when delivered by a route of administration by which the antibiotic agent by itself would not be effective. For example, including the potentiating compound in a therapeutic regimen may allow delivery of the first antibiotic by the oral route rather than the intravenous route. In certain embodiments of the invention including the potentiating agent in a therapeutic regimen reduces the dosing interval of the first antibiotic needed to achieve a desired therapeutic effect. For example, the antibiotic agent may be effective for a longer period of time in the presence of the potentiating agent. In certain embodiments of the invention including the potentiating agent in a therapeutic regimen allows the use of an antibiotic that is highly potent but too toxic for therapeutic use, *i.e.*, the potentiating agent allows a lower dose of the compound to be effective such that the antibiotic can be safely used without unacceptable side effects.

[00129] Inhibitors of microbial type II topoisomerases are exemplified herein as antibiotics for which antibiotic potentiator target genes and potentiating agents that modulate these target genes or their expression products can be identified. Quinolone antibiotics are of particular interest. The invention provides compositions containing an inhibitor of a microbial type II topoisomerase and an agent that potentiates activity of the inhibitor of a microbial type II topoisomerase. In certain embodiments the invention provides compositions comprising a quinolone antibiotic and an agent that potentiates activity of the quinolone.

[00130] However, the methods of the invention are applicable to any antibiotic class or particular antibiotic of interest. One of ordinary skill in the art will be able to select other classes of antibiotics for which potentiator target genes and/or potentiating agents can be

discovered using the inventive screening methods described herein. For example, the methods may be employed to identify genes whose deletion or functional inactivation potentiates the effects of antibiotics that are protein synthesis inhibitors, nucleic acid synthesis inhibitors, antibiotics that prevent synthesis of cell wall components, etc. The methods may also be employed to identify agents that potentiate any such antibiotic agent. One of ordinary skill in the art can, for example, consult *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th Ed., McGraw Hill, 2001, and Katzung, B. (ed.) *Basic and Clinical Pharmacology*, McGraw-Hill/Appleton & Lange; 8th edition (September 21, 2000), *Merck Manual of Diagnosis and Therapy*, 17th ed., *Physician's Desk Reference*, etc., to select other antibiotic agents for which potentiator target genes and/or potentiating agents may be sought. Specific examples of antibiotic classes of interest include aminoglycosides, lactams (*e.g.*, beta-lactams), and peptide antibiotics (*e.g.*, cyclic peptides).

[00131] Considerable effort has been devoted to discovering bacterial targets for the development of novel antibiotic agents. However, attention has largely been focused on identifying essential bacterial genes and attempting to discover agents that inhibit these genes or their expression products, based on the premise that agents that target such genes and/or their expression products would effectively inhibit bacterial growth. (see, *e.g.*, Rational identification of new antibacterial drug targets that are essential for viability using a genomics-based approach. Pharmacol Ther., 2002, 95: 1-20, 2002; DeVito *et al.*, Nat Biotechnol., 2002, 20: 478-483).

[00132] The present invention encompasses the recognition that this approach overlooks many potential target genes that may not be essential, at least not under typically used standard laboratory culture conditions. Among other advantages, the present invention identifies sets of microbial target genes that may not be essential under standard laboratory culture conditions but whose functional inactivation renders bacteria hypersensitive to an antibiotic, *e.g.*, the bacteria in which the gene is inactivated or that otherwise underexpress the gene are sensitive to the antibiotic at concentrations at which they would not otherwise be sensitive. In accordance with the invention, agents that inhibit with expression or activity of one or more of these target genes or their expression product(s), potentiate the antibiotic.

[00133] The invention is based in part on the identification of bacterial genes whose functional inactivation (or "knockout") render the bacterium more sensitive to antibiotics (*e.g.*, quinolones) than are otherwise identical bacteria in which the gene is not inactivated. Inactivation of these genes therefore potentiates the activity of the antibiotics (*e.g.*, quinolones). These genes are targets for the development of agents that potentiate the activity of antibiotics and are therefore referred to herein as "antibiotic potentiator target genes". The invention provides methods for identifying antibiotic (*e.g.*, quinolone) potentiator target gene. The invention further provides methods for identifying agents that modulate (*e.g.*, activate or inhibit) expression of an antibiotic potentiator target gene and methods for identifying agents that increase or inhibit an expression product of an antibiotic potentiator target gene.

[00134] One common means by which bacteria are intrinsically resistant to or acquire resistance to an antibiotic is by expressing or overexpressing an efflux pump, transporter, or porin that actively removes or allows removal of the antibiotic from the cell. Mutations in genes that encode certain of these proteins can also contribute to resistance in some cases. A number of such pumps, transporters, and porins are known, and their ability to mediate resistance to various antibiotics has been documented. A few examples of genes encoding subunits of such pumps, transporters, or porins are *tolC*, *acrA*, *acrB*, *ompC*, *oprM*, *norA*, *mexA*, and *mexB*. It has been postulated and in some cases demonstrated that agents that block or interfere with such pumps, transporters, or porins would reduce resistance and/or reduce the concentration of an antibiotic needed to inhibit bacterial growth and/or survival (See, *e.g.*, A.Y. Coban *et al.*, Chemotherapy, 2004, 50: 22-26; J. Kriengkauykiat *et al*, Antimicrob Agents Chemother., 2005, 49: 565-570; see J.M. Pages *et al.*, Trends Mol Med., 2005, 11: 382-389, and B. Marquez, Biochimie, 2005, 87: 1137-1147, for reviews)

[00135] In certain embodiments of the invention a target gene identified according to the inventive methods does not encode a subunit of an efflux pump, transporter, or porin. In certain embodiments of the invention an antibiotic potentiating agent does not act solely or primarily by binding to, inhibiting, or interfering with expression or activity of a bacterial efflux pump, transporter, porin, or subunit of any of these. The antibiotic potentiating agent may bind to or at least partially inhibit with expression of a bacterial efflux pump, transporter, porin, or subunit thereof. However, in the substantial absence of the particular efflux pump, transporter, porin, or subunit, the antibiotic potentiating agent would still significantly potentiate the antibiotic, thereby indicating that the agent does not act solely or primarily by binding to, inhibiting, or interfering with expression or activity of a bacterial efflux pump, transporter, porin, or subunit of any of these. Of course an inventive composition could contain an agent that binds to, inhibits, or interferes with expression or activity of a bacterial efflux pump, transporter, porin, or subunit of any of these in addition to a potentiating agent that acts against a target other than a bacterial efflux pump, transporter, porin, or subunit.

[00136] An antibiotic for which antibiotic potentiator target genes and potentiating agents may be discovered using the methods of the present invention and/or that may be potentiated using an antibiotic potentiating agent of the invention is typically a member of a class of agents, *e.g.*, a class of chemical compounds having similar structural features. Exemplary classes of antibiotics wherein members of the class share one or more structural features include, but are not limited to, quinolones, β-lactams (*e.g.*, penicillins or cephalosporins), carbapenems, aminoglycosides, macrolides, ketolides, tetracyclines, glycycyclines, lincomycins, oxazolidinones, amphenicols, ansamycins, polymyxins, aminomethlycyclines, lincosamides, streptogramins, 2,4-diamino-pyrimidines, nitrofurans, sulfonamides, sulfones, rifabutins, dapsones, peptides, and glycopeptides. In accordance with the invention, an agent that potentiates the antibiotic may be used to potentiate other antibiotics in the same structural class. For example, the antibacterial agent may be a quinolone antibiotic. Thus, in a nonlimiting example, an agent that potentiates a first quinolone antibiotic may be used to potentiate a second quinolone antibiotic. More specifically, an agent that potentiates a first fluoroquinolone such as norfloxacin may be used to potentiate a second fluoroquinolone such as ciprofloxacin. Of course it will be appreciated that an agent that potentiates any particular antibiotic in a class of agents need not potentiate all, most, or any other members of that class, although in certain embodiments of the invention the agent does potentiate at least one, many, most, or all other members of that class.

[00137] The antibiotic may have a known mechanism of antimicrobial action and/or may bind to and/or inhibit one or more bacterial target molecules or macromolecular complexes containing a bacterial target molecule. Mechanisms of action may include inhibiting or interfering with a biological or biochemical pathway of the bacterium. Exemplary pathways include, but are not limited to, protein synthesis, cell wall synthesis, DNA replication, transcription, cell division. It will be appreciated that biological and biochemical pathways are not mutually exclusive and that some biological or biochemical pathways may be considered to be subsets or sub-pathways of other biological or biochemical pathways. Mechanisms of action include, but are not limited to, inhibiting protein synthesis (*e.g.*, by binding ribosomal RNA or proteins, blocking tRNA binding to ribosome-mRNA complex, inhibiting peptidyl transferase), inhibiting or interfering with synthesis of a cell wall component (*e.g.*, inhibition of peptidoglycan synthesis, disruption of peptidoglycan cross-

linkage, disruption of movement of peptidoglycan precursors, disruption of mycolic acid or arabinoglycan synthesis), cell membrane disruption, inhibiting or interfering with nucleic acid synthesis or processing, acting as "antimetabolites" and either inhibiting an essential bacterial enzyme or competing with a substrate of an essential bacterial enzyme, inhibiting or interfering with cell division. In accordance with the invention, an agent that potentiates the antibiotic may be used to potentiate other antibiotics having the same or a similar mechanism of action. For example, the antibiotic may inhibit with bacterial protein synthesis. Thus, in a nonlimiting example, an agent that potentiates a first antibiotic that interferes with bacterial protein synthesis by binding to ribosomal RNA may be used to potentiate a second antibiotic that inhibits or interferes with protein synthesis by binding to ribosomal RNA. Of course it will be appreciated that an agent that potentiates any particular antibiotic having a particular mechanism of action need not potentiate all, most, or any other members having that mechanism of action, although in certain embodiments of the invention the agent does potentiate at least one, many, most, or all other antibiotics having that mechanism of action.

[00138] Molecules or macromolecular complexes containing them that may be targets of an antibiotic include, but are not limited to, peptidoglycans, penicillin binding proteins, lipopolysaccharides, ribosomes or ribosomal subunits or RNA or protein components thereof (23S rRNA, 16S rRNA, 5S rRNA, proteins of the 30S or 50S subunit), DNA-dependent DNA polymerase, DNA-dependent RNA polymerase, microbial type I topoisomerase, microbial type II topoisomerase (*e.g.*, topoisomerase IV or gyrase), enzymes involved in synthesis of essential cofactors or nucleic acid precursors (*e.g.*, nucleotides) such as dihydrofolate reductase, enzymes involved in cell division such as FtsZ, etc. In accordance with the invention, an agent that potentiates the antibiotic may be used to potentiate other antibiotics that bind to and/or inhibit expression or activity of the same or a structurally similar molecular target or macromolecular complex or may be used to potentiate other antibiotics that bind to and/or inhibit expression or activity of a molecular target that participates in the same biological or biochemical pathway. For example, the antibacterial agent may be an inhibitor of a bacterial type II topoisomerase, *e.g.*, an inhibitor of topoisomerase IV, gyrase, or both. In a nonlimiting example, an agent that potentiates a first antibiotic that binds to and/or inhibits or interferes with expression or activity of a bacterial type II topoisomerase may be used to potentiate a second antibiotic that binds to and/or inhibits or interferes with expression or activity of the same or a different type II

topoisomerase. More specifically, an agent that potentiates a quinolone antibiotic whose primary target is DNA gyrase may potentiate an antibiotic whose target is topoisomerase IV. Of course it will be appreciated that an agent that potentiates an antibiotic that has a particular molecular target need not potentiate all, most, or any other antibiotics having that target, a structurally similar target, or a target that participates in the same biological or biochemical pathway, although in certain embodiments of the invention the agent does potentiate at least one, many, most, or all other antibiotics having that target, a structurally similar target, or a target that participates in the same biological or biochemical pathway.

[00139] The target genes of the invention may be found in bacteria of any one or more types, *e.g.*, Gram negative bacteria, Gram positive bacteria, and/or acid fast bacteria. Any of these bacteria may therefore be used in the methods for identifying a target gene described herein, and any target gene can be used to identify agents that potentiate antibiotics (*e.g.*, quinolone antibiotics). One of ordinary skill in the art will appreciate that it may be convenient to perform the target gene identification methods using a bacterial species or strain that is commonly used in the laboratory and for which reagents and methods for performing microbiological and/or genetic manipulation are well developed and widely available. Many such strains are known to one of skill in the art. It may be desirable to utilize a non-pathogenic strain of bacteria to perform the target gene identification methods, wherein the strain is a member of bacterial species or genus that contains pathogenic members. Once a target gene is found in any particular bacterial strain, species, or genus, homologs can be readily identified in one or more other bacterial strains, species, or genuses, as discussed further below. Such homologs can be used in the methods for identifying an antibiotic potentiating agent, *e.g.*, a quinolone potentiating agent. Furthermore, an antibiotic potentiating agent identified according to a method that involves a target gene from a particular bacterial genus, species, or strain, is of use to inhibit homologous target genes in other bacterial genuses, species, or strains, and is therefore of use to potentiate the antibiotic when used to inhibit growth and/or survival of bacteria of a genus, species, or strain that contains the homologous target gene.

[00140] Suitable organisms from which target genes can be identified and/or against which potentiating agents can be used can include, but are not limited to members of the following genuses: *Actinomyces, Staphylococcus, Streptococcus, Enterococcus, Erysipelothrix, Neisseria, Branhamella, Listeria, Bacillus, Corynbacterium, Erysipelothrix, Gardnerella, Mycobacterium, Nocardia, Enterobacteriaceae, Escherichia, Salmonella,*

Shigella, Yersinia, Enterobacter, Klebsiella, Citrobacter, Serratia, Providencia, Proteus, Morganella, Edwardsiella, Erwinia, Vibrio, Aeromonas, Helicobacter, Campylobacter, Eikenella, Pasteurella, Pseudomonas, Burkholderia, Stenotrophomonas, Acinetobacter, Ralstonia, Alcaligenes, Moraxella, Mycoplasma, Legionella, Francisella, Brucella, Haemophilus, Bordetella, Clostridium, Bacteroides, Porphyromonas, Prevotella, Fusobacterium, Borrelia, Chlamydia, Rickettsia, Ehrlichia, Bartonella, Trichomonas, and *Treponema.*

[00141] In particular embodiments of the invention the bacteria are species that are causative agents of disease in humans and/or animals. Examples include, but are not limited to, *Aeromonas hydrophila, Bacillus subtilis, Escherichia coli, Enterobacter cloacae, Campylobacter jejuni, Haemophilus influenzae, Klebsiella pneumoniae, Klebsiella oxytoca, Legionella pneumophila, Pasteurella multocida, Proteus mirabilis, Proteus vulgaris, Morganella morganii, Helicobacter pylori, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Salmonella enterica, Salmonella typhimurium, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes,* and *Streptococcus agalactiae.*

[00142] In certain embodiments of the invention the target genes are found in a bacterial species that is sensitive to a bacterial type II topoisomerase inhibitor such as a quinolone antibiotic. In certain embodiments of the invention the target genes are found in a bacterial species or strain that displays intrinsic or acquired resistance to a bacterial type II topoisomerase II inhibitor, such as a quinolone antibiotic. In certain embodiments of the invention the target genes are found in a bacterial species that is sensitive to an antibiotic that binds to the bacterial 30s ribosomal subunit such as an aminoglycoside antibiotic. In certain embodiments of the invention, the target genes are found in a bacterial species or strain that displays intrinsic or acquired resistance to an antiobiotic that binds to the bacterial 30s ribosomal subunit such as an aminoglycoside antibiotic.

[00143] The invention provides methods and reagents that may be used to identify agents that either directly or indirectly modulate expression and/or activity of a target gene. The methods include cell-free assays, cell-based assays, and computational methods.

[00144] The invention also provides agents that modulate, e.g., inhibit, expression of a antibiotic potentiator target gene. The invention provides agents that modulate activity of an expression product of an antibiotic potentiator target gene, *e.g.*, that inhibit or interfere with activity of an expression product of an microbial antibiotic potentiator target gene. An agent that modulates activity of an expression product of target gene can do so directly, *i.e.*, by a mechanism that involves a physical interaction of the agent with the expression product. Alternatively or additionally, an agent can modulate activity of an expression product of a target gene indirectly, *i.e.*, by a mechanism that does not involve a physical interaction with the expression product of the target gene. For example, the agent can directly or indirectly modulate an enzyme that is at least in part responsible for synthesizing a substrate of or cofactor for the expression product or is responsible for modifying or activating the expression product. In a nonlimiting example, if a first and second polypeptide are subunits of a protein, then an agent that binds to the second polypeptide may prevent or hinder interaction of the two polypeptides, and may thus indirectly modulate activity of the first polypeptide. Thus, antibiotic potentiating agents of the present invention are not limited to agents that modulate expression of a target gene or physically interact with an expression product of a target gene but encompass any agent that direct or indirectly interferes with expression of the target gene and/or interferes with activity of an expression product of the gene.

II. Antibiotics

[00145] A large variety of classes of antibiotics can be used according to the present invention. Exemplary structural classes of antibiotics include, but are not limited to, aminoglycosides, aminomethylcyclines, amphenicols, ansamycins, β-lactams (*e.g.*, penicillins or cephalosporins), carbapenems, dapsones, 2,4-diaminopyrimidines, glycopeptides, glycycyclines, ketolides, lincomycins, lincosamides, macrolides, nitrofurans, oxazolidinones, peptides, polymyxins, quinolones, rifabutins, streptogramins, sulfonamides, sulfones, tetracyclines, and combinations thereof. A few examples of classes of antibiotics are described below.

Quinolone Antibiotics and Other Bacterial Type II Topoisomerase Inhibitors

[00146] Quinolone antibiotics are compounds that contain a quinolone or a naphthyridine nucleus with any of a variety of different side chains and substituents. Numerous modifications of the originally identified core structures have been made resulting in a large number of compounds with activity against differing groups of bacteria. Quinolone antibiotics are described, *e.g.*, in Ronald, A.R. and Low, D.E., (eds.), "*Fluoroquinolone Antibiotics*", Birkhäuser Verlag, Basel, 2003; DaSilva, AD, *et al.*, "Biological activity and synthetic methodologies for the preparation of fluoroquinolones, a class of potent antibacterial agents", *Curr Med Chem* 10(1):21-39, 2003; Van Bambeke, F., *et al.*, "Quinolones in 2005: an update", *Clin Microbiol. Infect.*, 11: 256-280, 2005; U.S. Pat. Nos. 3,669,965); 4,563,459; 4,620,007; 4,382,892; 4,985,557 5,053,407; and 5,142,046). Figure 9 depicts the core structures and numbering system of classical quinolone antibiotics (4-quinolone and 4-naphthyridine systems). It is noted that the numbering system shown herein is used for purposes of convenience and is not intended to be limiting. The invention encompasses quinolone compounds in which an alternative numbering system is used.

[00147] Quinolone antibiotics include, but are not limited to, any of the antibacterial agents disclosed in the foregoing references including, but not limited to, ciprofloxacin, oxolinic acid, cinoxacin, flumequine, miloxacin, rosoxacin, pipemidic acid, norfloxacin, enoxacin, moxifloxacin, gatifloxacin, ofloxacin, lomefloxacin, temafloxacin, fleroxacin, pefloxacin, amifloxacin, sparfloxacin, levofloxacin, clinafloxacin, nalidixic acid, enoxacin, grepafloxacin, levofloxacin, lomefloxacin norfloxacin, ofloxacin, trovafloxacin, olamufloxacin, cadrofloxacin, alatrofloxacin, gatifloxacin, rufloxacin, irloxacin, prulifloxacin, pazufloxacin, gemifloxacin, sitafloxacin, tosulfloxacin, amifloxacin, nitrosoxacin-A, DX-619, and ABT-492. Quinolone antibiotics include fluoroquinolones (*e.g.*, having a fluorine substituent at the C-6 position), and non-fluoroquinolones. Also included within the scope of quinolone antibiotics are derivatives in which a quinolone is conjugated with, *e.g.*, covalently bound to, another core structure. For example, U.S. Pub. No. 2004-0215017 discloses compounds in which an oxazolidinone, isoxazolinone, or isoxazoline is covalently bonded to a quinolone.

[00148] Included within the scope of quinolone antibiotics that can be potentiated by inactivation of the quinolone potentiator target genes or their expression products are compounds that have a core structure related to the 4-oxo-1,4-dihydroquinoline and 4-oxo-1,4 dihydronapthyridine systems, *e.g.*, 2-pyridones, 2-naphthyridinones, and benzo[b]napthyridones. 2-pyridones are potent inhibitors of bacterial type II topoisomerases (Shen, LL, et al., *Curr. Pharm. Des.*, 3:169-176; Saiki, AYC, et al., *Antimicrob. Agents Chemother.*, 43: 1574-1577, 1999). The core structures are depicted in Figure 9.

[00149] Also included within the scope of quinolone antibiotics that can be potentiated are compounds that have core structures related to the quinolone core structures depicted in Figure 9 or 10. Certain of these core structures are shown in Figures 11A and 11B, and references thereto are provided in Ronald, *supra*. The invention encompasses the use of quinolone potentiating agents identified as described herein to potentiate these antibiotics.

[00150] In addition to the quinolone antibiotics, a variety of agents are known in the art that inhibit one or more bacterial type II topoisomerase inhibitors, some of which are structurally related to quinolones. Exemplary inhibitors that bind to GyrB include the coumarins, novobiocin and coumermycin A1, cyclothialidine, cinodine, and clerocidin. Additional compounds that are reported to bind to and/or inhibit gyrase, topoisomerase IV, or both, are disclosed in U.S. Pat. Nos. 6,608,087 and 6,632,809 and in U.S. Pub. Nos. 20040043989 and 20050054697. The present invention encompasses the use of quinolone potentiating agents to potentiate any of these agents, *e.g.*, for use in combination with any of these compounds or any compound that inhibits one or more microbial type II topoisomerases.

[00151] Quinolones exert their antibacterial effects at least in part by targeting bacterial type II topoisomerases, namely gyrase and topoisomerase IV, enzymes that catalyze breakage and rejoining of DNA strands during normal cell growth. DNA gyrase exists as an A_2B_2 heterotetramer, encoded by the *gyrA* and *gyrB* genes. Topoisomerase IV exists as a C_2E_2 heterotetramer, encoded by the *parC* and *parE* genes. It is noted that the foregoing nomenclature, while applicable to *E. coli* and many other microorganisms, is not universal. For example, the *parC* and *parE* are sometimes referred to as *grlA* and *grlB* respectively in Gram positive bacteria. These enzymes are conserved among Gram negative and positive bacteria and are essential for viability. DNA gyrase introduces negative coils into DNA and can remove both negative and positive supercoils. The main function of the topoisomerase IV is the decatenation of interlinked replicated chromosomes, allowing the partitioning daughter chromosomes following DNA replication.

[00152] Quinolones act by forming a ternary complex with the topoisomerase enzymes and DNA. The lethal effect may result due to enhancement of DNA cleavage and/or by blocking DNA religation following cleavage by the topoisomerase rather than primarily from the inhibition of DNA replication. Quinolones increase the intracellular concentrations of the cleavage complexes that are intermediates in the topoisomerase-mediated reactions. The accumulation of permanent double-stranded DNA breaks eventually leads to bacterial death. The affinity of many quinolone antibiotics and derivatives differs towards DNA gyrase and DNA topoisomerase IV (see, *e.g.*, Drlica and Hooper, 2003). Resistance to quinolones arises primarily due to a variety of mutations which make the enzymes less sensitive to quinolones or which affect microbial efflux pumps and decrease cellular accumulation of the drug. Amino acids at which mutations are particularly likely to result in resistance have been identified. Portions of the enzyme containing such amino acids have been denoted quinolone resistance determining regions. See, *e.g.*, Ronald, AL, *supra.* Quinolone resistance can arise in a step-wise fashion as bacteria accumulate multiple mutations in either the same or different type II topoisomerase subunits. The invention contemplates use of a quinolone potentiating agent in combination with a quinolone against bacteria having one or more mutations that confer quinolone resistance.

Aminoglycosides

[00153] Aminoglycosides are a group of antibiotics that are effective against certain types of bacteria. Examples of aminoglycosides include amikacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin, tobramycin, ad apramycin. Those which are derived from *Streptomyces* genus are named with the suffix –mycin, while those which are derived from *micromonospora* are named with the suffix –micin.

[00154] Aminoglycosides work by binding to the bacterial 30S ribosomal subunit (some work by binding to the 50s subunit), inhibiting the translocation of the peptidyl-tRNA from the A-site to the P-site and also causing misreading of mRNA, leacing the bacterium unable to synthesize proteins vital to its growth. However, the exact mechanism of action of aminoglycosides in not fully known.

[00155] Aminoglycosides are useful primarliy in infections involving aerobic, Gram-negative bacteria, such as *Pseudomonas, Acinetobacter*, and *Enterobacter.* In addition, some mycobacteria, including the bacteria that cause tuberculosis, are susceptible to aminoglycosides. The most frequent use of aminoglycosides is empiric therapy for serious infections such as septicemia, complicated intra-abdominal infections, complicated urinary tract infections, and nosocomial respiratory tract infections. Usually, once cultures of the causal organism are grown and their susceptibilities tested, aminoglycosides are discontinued in favor of less toxic antibiotics.

[00156] Streptomycin was the first effective drug in the treatment of tuberculosis, though the role of aminoglycosides such as streptomycin and amikacin has been eclipsed (because of their toxicity and inconvenient route of administration) except for multiple drug resistant strains.

[00157] Infections caused by Gram-positive bacteria can also be treated with aminoglycosides, but other types of antibiotics are more potent and less damaging to the host. In the past, the aminoglycosides have been used in conjunction with beta-lactam antibiotics in streptococcal infections for their synergistic effects, particularly in endocarditis. One of the most frequent combinations is Ampicillin (a beta-lactam or penicillin-related antibiotic) and Gentamicin.

[00158] Because of their potential for ototoxicity and nephrotoxixity (kidney toxicity), aminoglycosides are administered in doses based on body weight. Blood drug levels are used to estimate how well the kidneys are functioning and as a marker for kidney damage caused by these drugs.

Peptide Antibiotics

[00159] Over 400 natural antimicrobial peptides have been isolated and characterized. Based on chemical structure, these peptides may be classified into two main groups: linear and cyclic (R.E. Hancock *et al.*, Adv. Microb. Physiol., 1995, 37: 135-137; H. Kleinkauf et al., Criti. Rev. Biotechnol., 198, 8: 1-32; D. Perlman and M. Bodansky, Annu. Rev. Biochem., 1971, 40: 449-464. The mode of action for the majority of these peptides (both linear and cyclic) is believed to involve membrane disruption, leading to cell leakage (A. Mor, Drug Develop. Res., 2000, 50: 440-447). The linear peptides, such as magainins and melitting, exist mainly as a-helical amphipathic structures (containing segregated hydrophobic and hydrophilic moieties), or as β-helices as found in gramicidin A (GA). Cyclic peptides, which mainly adopt an amphipatic β-sheet structures can be further divided into two subgroups: those containing disulfide bonds, such as tachyplesin, and those that do not, such as gramicidin S (D. Audreu and L. Rivas, Biopolymers, 1998, 47: 415-433).

[00160] Peptide antibiotics also fall into two classes: non-ribosomally synthesized peptides, such as the gramicicins, polymyxins, bacitracins, glycopeptides, etc., and ribosomally synthesized (natural) peptides. The former are often drastically modified and are largely produced by bacteria, whereas the latter are produced by all species of life (including bacteria) as a major component of the natural host defense molecules of these species.

[00161] In certain embodiments, the peptide antibiotic is a lipopeptide antibiotic such as colistin, daptomycin, surfactin, friulimicin, aculeacin A, iturin A, and tsushimycin.

[00162] Colistin (also called Colimycin) is a polymixin antibiotic discovered more than 50 years ago. It is a cyclic lipopeptide antibiotic which penetrates the cell wall of Gram negative bacteria by self-induced mechanism by chelating divalent ions. Colistin destabilizes the wall and can insinuate into it. Colistin basically perforates the cell wall, causing distortion of this structure and the release of intracellular constituents. Increasing multidrug resistance in Gram-negative bacteria, in particular *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*, presents a critical problem. Limited therapeutic options have forced infectious disease clinicians and microbiologists to reappraise the clinical application of Colistin. Colistin is associated with neurotoxicity and nephrotoxicity. Dosage regimen and novel formulation may be an answer to address the toxicity issue.

III. Target Genes and Methods for their Identification

[00163] The invention provides methods for identifying an antibiotic potentiator target gene. Certain of the methods involve determining whether complete or partial functional inactivation of a gene, *e.g.*, by deletion or other genetic alteration that eliminates or greatly reduces expression of the gene or results in a gene product that substantially lacks functional activity (*e.g.*, displays less than about 10% of the activity that would be exhibited in the absence of the genetic alteration, preferably less than about 5% of the activity) or by inhibiting an expression product of the gene, renders a microbial cell more sensitive to the antibiotic relative to cells in which the expression product of the gene has greater activity. Other methods involve determining whether overexpression of a gene, *e.g.*, relative to wild type levels, confers increased resistance to the effect of an antibiotic.

[00164] A major concept of the invention is to compare the antibiotic sensitivity of microbial cells that have differing levels of functional activity of a gene product and to either qualitatively or quantitatively determine whether sensitivity to the antibiotic agent depends at least in part on the level of functional activity of the gene product. Within this conceptual framework, a variety of different approaches may be employed. For example, antibiotic sensitivity of cells in which a gene is partly or completely functionally inactivated (*e.g.*, as a result of a genetic alteration such as partial or complete deletion of the gene) can be compared with sensitivity of cells that display higher levels of functional activity of the gene. Alternately, antibiotic sensitivity of cells that overexpress a particular gene can be compared with sensitivity of cells that express the gene at wild type levels or that

underexpress the gene. It will be appreciated that the comparison can be performed in a number of ways, and that it is not necessary to subject cells displaying different levels of functional activity of a gene to the antibiotic at the same time or in the same experiment. For example, once the sensitivity of cells exhibiting any particular level of functional activity of a gene product (*e.g.*, wild type cells) is determined, this information can be used as a basis for comparison in future experiments.

[00165] Any of a variety of specific methods may be used to determine whether functional inactivation of a gene confers hypersensitivity, and any such method is within the scope of the invention. One method of identifying an antibiotic potentiator target gene comprises steps of: (a) contacting a first microbial cell with an antibiotic, wherein the microbial cell lacks or has significantly reduced functional activity of an expression product of a gene relative to a second microbial cell; and (b) determining that the gene is an antibiotic potentiator target gene if the growth or survival of the first microbial cell is detectably lower than the growth or survival of the second microbial cell. It will be appreciated that the method typically involves comparing growth or survival that the first and second microbial cells would exhibit under substantially equivalent conditions, particularly with respect to the concentration of the antibiotic and the time of exposure. However, substantially equivalent conditions need not actually be employed in performing the method, provided that the growth or survival results for the first and second microbial cells can be correlated with what would be expected to occur under substantially equivalent conditions.

[00166] Substantially equivalent conditions of antibiotic exposure with respect to concentration of the antibiotic typically means that the concentration of antibiotic to which the first and second microbial cells are exposed are within a factor of 2-fold of one another, or that the concentrations would be expected to have substantially the same effect on identical cells (for example, two different concentrations that are both much larger than the MIC would be expected to have substantially the same inhibitory effect even if the absolute concentrations varied by more than a factor of 2, and two different concentrations that are both much smaller than the MIC would be expected to have substantially no effect even if the absolute concentrations varied by more than a factor of 2). Preferably, substantially equivalent antibiotic exposure is exposure at concentrations that differ by no more than a factor of 2. The concentrations may be identical within experimental error, or the larger concentration may be 110% or less, 120% or less, 130% or less, 140% or less, or 150% or less of the smaller concentration. Alternately, the concentrations may differ by 10% or less, 20% or less, 30% or less, 40% or less, or 50% or less of the MIC for the antibiotic. With respect to time during which exposure occurs, substantially equivalent conditions would typically mean that the length of exposure differs by no more than a factor of 2 and may, for example, differ by 10% or less, 20% or less, 30% or less, 40% or less, or 50% or less of the shorter time of exposure or be substantially identical (*i.e.*, identical to within 2% of the shorter time of exposure). Substantially equivalent conditions may also entail use of the same growth medium, temperature, etc., for cells whose growth or survival is to be compared.

[00167] The particular antibiotic concentration selected will depend on a variety of parameters including the bacterial species or strains used, whether growth or survival (viability following a period of exposure to the antibiotic) is to be assessed, etc. Typically, the concentration will be sublethal for a growth assay. The concentration may be one that does not significantly reduce bacterial growth but is sufficient to cause at least some alterations in bacterial physiology. For example, the concentration may be one that causes detectable alterations in expression of one or more genes. In certain embodiments of the invention the concentration selected for a screen employing a growth assay is between 1% and 5% of the MIC, between 5% and 10% of the MIC, between 10% and 25% of the MIC, between 25% and 50% of the MIC, between 50% and 75% of the MIC, between 75% and 95% of the MIC, or any specific subrange or value within a foregoing range. In certain embodiments of the invention the concentration selected for a screen employing a growth assay reduces growth of wild type bacteria (not having functional inactivation of a gene) to between 5% and 10%, between 10% and 25%, between 25% and 50%, between 50% and 75%, or between 75% and 95% of the growth in the absence of the antibiotic, or any specific subrange or value within a foregoing range. In certain embodiments of the invention the concentration selected for a screen employing a survival assay is between 1 and 2 times the MIC, between 2 and 5 times the MIC, or between 5 and 10 times the MIC.

[00168] Growth or survival can be assessed using cells growing in liquid media or on solid or semi-solid media. Any method known in the art can be used to determine whether an agent inhibits growth, proliferation, and/or survival. Examples include measuring optical density in liquid culture, measuring colony formation, or measuring bacterial viability. Bacterial viability can be assessed based on metabolic characteristics such as oxidation/reduction state, ability to metabolize particular substrate(s) or produce particular

metabolite(s), or based on membrane integrity, which can be detected by evaluating ability of a bacterial cell to exclude a particular substance such as a detectable molecule (*e.g.*, a fluorescent or luminescent molecule) from the cell interior. In one embodiment, a commercially available assay such as the LIVE/DEAD BacLight Bacterial Viability assay (Molecular Probes, now owned by Invitrogen, Carlsbad, CA) is used. This assay utilizes mixtures of SYTO® 9 green fluorescent nucleic acid stain and the red fluorescent nucleic acid stain, propidium iodide. These stains differ both in their spectral characteristics and in their ability to penetrate healthy bacterial cells. When used alone, the SYTO 9 stain labels bacteria with both intact and damaged membranes. Propidium iodide, however, penetrates only bacteria with damaged membranes, competing with the SYTO 9 stain for nucleic acid binding sites when both dyes are present. When mixed in recommended proportions, SYTO 9 stain and propidium iodide produce green fluorescent staining of bacteria with intact cell membranes and red fluorescent staining of bacteria with damaged membranes. The background remains virtually nonfluorescent. The ratio of green to red fluorescence intensities therefore provides a quantitative index of bacterial viability. A fluorometer can be used to detect the fluorescence intensities. Another suitable assay for determining the number of viable bacterial cells in culture is based on quantitation of the ATP present. ATP is an indicator of metabolically active cells. The BacTiter-Glo™ Assay (Promega, Madison, WI) is a commercially available assay based on this principle that involves adding a single reagent (BacTiter-Glo™ Reagent) directly to bacterial cells in medium and measuring luminescence. Many additional assays suitable for assessing bacterial viability are described in "Handbook of Fluorescent Probes and Research Products" (Molecular Probes, 9th edition, 2002) and "The Handbook — A Guide to Fluorescent Probes and Labeling Technologies" (Invitrogen, 10th edition, available at the Invitrogen web site). In certain embodiments of the invention the assay is performed in a high throughput format.

[00169] The methods of the invention advantageously make use of a collections of bacterial strains in which a different gene is altered in each strain. Typically the strains will be members of a bacterial species, e.g., *E. coli* or S. *aureus* and will be genetically identical except for the genetic alteration. The collection of strains will advantageously include members with alteration or deletion of substantially all non-essential genes of the bacterium, with each member having an alteration or deletion of a different gene. The alteration may, for example, involve deletion of all or part of the gene, so that either (i) no functional gene product is synthesized; (ii) the amount of functional gene product is substantially reduced; or

(iii) the gene product has substantially reduced or no activity. The availability of complete genome sequences for a variety of different bacteria has facilitated the development of such strain collections. Deletion or functional inactivation can be achieved using a variety of different methods known in the art.

[00170] In other embodiments a collection of bacterial strains in which a different gene is overexpressed in each strain is used. Overexpression can be achieved, *e.g.*, by introducing an expression vector containing the relevant gene (or the coding portion thereof) into the cell. Either the naturally occurring promoter or a heterologous promoter can be used. The vector can be a multicopy plasmid, thereby providing many copies of the gene. Alternately or additionally, a strong promoter functional in bacterial cells could be used to express the gene at high levels. A recombinant nucleic acid construct encoding the gene can be integrated into the bacterial chromosome. It will be appreciated that the sequence of the gene introduced into the cells may differ from the wild type sequence but will typically have a high degree of nucleotide similarity to it (*e.g.*, at least 80%, preferably at least 90%, and preferably close to 100% identity). In some embodiments a gene derived from one bacterial species is introduced into a different bacterial species.

[00171] In some embodiments of the invention the members of the strain collection express genes under the control of a regulatable promoter. A regulatable promoter is one for which transcription of an operatively linked nucleic acid sequence is induced by an agent such as an inducer, or repressed by an agent such as a repressor, or induced or repressed by a condition such as heat. A variety of regulatable promoters that function in bacterial cells are known in the art. Typical inducers and repressors include small molecules such as arabinose, isopropyl-beta-thiogalactopyranoside (IPTG), tetracycline and xylose. An exemplary regulatable promoter is the arabinose-regulated P_{bad} promoter (L-M. Guzman, et al., *J. Bacteriology*, 177: 4121-4130, 1995), Other inducible/repressible single or multi-plasmid bacterial expression systems are based on the *lac* promoter, hybrid *lac* promoter, or the tetracycline response element, and variants thereof. Examples of such expression systems include the PLtetO-1 (tetracycline-inducible) system & PLlacO-1 (IPTG-inducible) system (R. Lutz & H. Bujard, *Nucleic Acids Research*, 25: 1203-1210, 1997). See also U.S. Patent Nos. 4,952,496 and 6,436,694.

[00172] Methods for generating strains suitable for use in the methods of the invention are known in the art. For example, recombinant nucleic acid constructs can be made and introduced into bacterial cells as described, *e.g.*, in *Current Protocols in Molecular Biology*,

supra, and Sambrook, *supra.* Methods for performing gene disruption in various bacteria such as *E. coli* and *S. pneumoniae* are described, *e.g.*, in (Datsenko *et al.*, 2000, Proc. Natl. Acad. Sci. USA 97: 6640-5; Murphy, 1998, J. Bacteriol. 180: 2063-71; and Winans *et al.*, J. Bacteriol 161: 1219-21; Link *et al.*, 1997, J. Bacteriol. 179: 6228-37; Arigoni *et al.*, 1998, Nat. Biotechnol. 16(9): 851-6; Akerley *et al.*, Proc. Natl. Acad. Sci USA 95: 8927-32; Lee *et al.*, 1999 Appl. Environ. Microbiol. 65: 1883-90 and Lee *et al.*, 1998, Appl. Environ Microbiol. 64: 4796-802). Methods for obtaining chromosomal integration of nucleic acid constructs are also known, and have been described, for example in, Gerhardt *et al.*, *supra*; Link *et al.*, 1997, J. Bacteriol. 179: 6228-37; and Metcalf *et al.*, 1996, Plasmid 35: 1-13.

Quinolone Potentiator Target Genes

[00173] As described in Example 1, a first set of target genes was identified by exposing members of a collection of bacterial strains to a sublethal concentration of a quinolone. Each strain had a genetic alteration resulting in functional inactivation of a different gene. The sublethal concentration was selected so that it did not significantly impair bacterial growth but was sufficient to cause alterations in gene expression, as determined by microarray analysis, thereby confirming that the concentration was sufficient to cause alterations in bacterial physiology. Bacterial strains that failed to grow in the presence of the quinolone were identified. A second screen was performed (Example 2), in which the ability of the strains to survive following exposure to a lethal concentration of a quinolone antibiotic was assessed.

[00174] In certain embodiments of the invention the quinolone potentiator target gene encodes a DNA metabolizing enzyme, by which is meant any polypeptide that chemically modifies, synthesizes, degrades, cleaves, processes, and/or unwinds or otherwise alters the topology of DNA. Examples include DNA-dependent DNA polymerases, endonucleases, exonucleases, helicases, primases, recombinases, etc.

[00175] In embodiments of particular interest the quinolone potentiator target gene encodes a helicase, helicase subunit, or a helicase-like polypeptide.

[00176] In certain embodiments of the invention the quinolone potentiator target gene encodes an enzyme that plays a role in the microbial fatty acid synthesis pathway.

[00177] In certain embodiments of the invention the quinolone potentiator target gene encodes a transcription factor. In other embodiments of the invention the quinolone

potentiator target gene does not encode a transcription factor. For example, in certain embodiments of the invention the gene is not *dksA* or a homolog thereof.

[00178] In certain embodiments of the invention functional inactivation of the quinolone potentiator target gene reduces the MIC of a quinolone antibiotic by at least a factor of 5, 10, 50, 100, 500, 10^3, 5×10^3, 10^4, 5×10^4, or more.

[00179] A target gene of the present invention may have one or more of the features described herein, provided that such features are not mutually inconsistent.

[00180] The invention provides a first set of target genes consisting of genes whose functional inactivation resulted in strains that either (i) failed to grow in the presence of a sublethal, non-inhibitory concentration of a quinolone antibiotic; (ii) failed to survive a period of exposure to a lethal concentration of a quinolone antibiotic; or (iii) both failed to grow in the presence of a sublethal, non-inhibitory concentration of a quinolone antibiotic and failed to survive a period exposure to a lethal concentration of a quinolone antibiotic. The set of target genes includes *recA, recB, recC, recG, recN, ruvA, ruvB, ruvC, uvrD, xerD, xseA, xseB, pinQ, dnaT, priA, polA, thyA, b1440, fabH, tolC, acrA, acrB, ompC, dksA, fis, dapF, fliN, jw5303, yhfT, yqgC, ycjS, resA, dapF, ygoC, rimK,* and *yicN.* The invention further provides subsets of these genes. A first subset consists of genes whose deletion resulted in cells that both failed to grow in the presence of a sublethal, non-inhibitory concentration of a quinolone antibiotic and failed to survive a period exposure to a lethal concentration of a quinolone antibiotic. A second subset consists of genes whose deletion resulted in cells that failed to grow in the presence of a sublethal, non-inhibitory concentration of each of two different quinolone antibiotics. A third subset consists of genes for which homologs exist in representative Gram positive and Gram negative organisms (*e.g.*, *E. coli* and *S. aureus*). The invention specifically includes each possible subset of the aforementioned target genes, including subsets in which any one or more of the target genes is explicitly excluded. In some embodiments, genes that encode a subunit of an efflux pump, transporter, or porin are excluded. In some embodiments, genes that encode a transcription factor or subunit thereof are excluded.

[00181] The present invention provides a subset of quinolone potentiator target genes including *dapF, fabH, fis, fliN, jw5303, priA, recA, recB, recC, recG, resA, ruvA, ruvB, ruvC, ruvC, uvrD, ycjS,ydfL, yhfT,* and *yqgC.*

[00182] In addition to the quinolone potentiator target genes identified in the screens, the invention also contemplates that other genes functioning in the same biological or biochemical pathway as any one or more of these genes are also potential targets for quinolone potentiation. Specific quinolone potentiator target genes and biological pathways of particular interest are described further below.

[00183] In certain embodiments of the invention the quinolone potentiator target gene encodes a helicase, helicase subunit, or helicase-like polypeptide. Examples of such genes identified in the inventive screen include *recA*, *recB*, *recC*, *recG*, *ruvA*, *ruvB*, *ruvC*, *priA*, *dnaT*, *pinQ* and *uvrD*. Helicases are a group of enzymes that catalyze the separation of duplex nucleic acids into single strands in an ATP dependent process, hence allowing other critical cellular processes to take place. These enzymes have been classified into 5 major groups that share a conserved "helicase" structural module. This module is related to the structure of the bacterial RecA protein and is recognized in the art. It contains five contiguous parallel β strands and their tandem α helices. Within this module there are a number of motifs recognized in the art as characteristic of helicases including the Walker A and B motifs, which are involved in nucleotide interactions, *e.g.*, with ATP. See, *e.g.*, Story and Steitz, 1995; Caruthers, 2002, and references therein. For purposes of this invention any polypeptide that possesses the characteristic RecA-like core structure as described in Caruthers, 2002, and catalyzes the separation of duplex nucleic acids into single strands in an ATP-dependent manner will be termed "helicases" while polypeptides that possess the characteristic RecA-like structure but that do not possess the aforementioned activity will be termed "helicase-like".

[00184] The RecA protein is a key sensor and activator in response to DNA damage and plays a major role in inducing the SOS response pathway following such damage. It is known that RecA is also involved in other cellular processes in addition to recombination and DNA damage repair. A fundamental event in both homologous recombination and SOS response induction is the formation of a RecA-ssDNA-ATP nucleoprotein filament. In this conformation RecA acts as both a recombinase and co-protease. In the latter function it activates the SOS response by cleaving the LexA repressor protein, which results in the induction of genes that are repressed by LexA under normal conditions. Over 30 SOS genes, and UmuD, a sub-unit of polymerase IV, also involved in the SOS response are induced (Courcelle and Hanawalt, 2003; Sutton et al., 2000). Another fundamental role of RecA is to maintain the integrity of the genetic material. The binding of RecA to single-stranded DNA

regions that block replication forks serves as the sensor that replication is blocked and maintains the integrity of the replication fork itself until replication can resume (Courcelle, 2003). Thus one biological pathway of particular interest herein is recovery of arrested DNA replication forks. The invention implicates genes whose expression products play a role in this pathway as quinolone potentiator target genes. The invention implicates genes whose expression and/or activity is upregulated by RecA as quinolone potentiator target genes.

[00185] The RecA protein is highly conserved and is cross species functional. For example, *recA* homologs from *Yersinia pestis, Bacillus anthracis* and *M. tuberculosis* have been shown to complement the *E. coli recA*-mutation (Suchkov and Mishan'kin, 1989; Ko *et al.*, 2002; Nair and Steyn, 1991). Thus inhibitors of RecA identified using the RecA protein from one species would be expected to potentiate quinolone activity in a wide variety of bacteria.

[00186] The RecA protein has many functional features that present points of intervention to inhibit its activity in accordance with the present invention. Multiple alignment of the sequence reveals a canonical structure of RecA-like proteins consisting of distinct segments or motifs (Figure 1). These segments or modules are highly conserved and have been assigned functional roles based on genetic, biochemical and structural studies. Such modules are involved in DNA damage recognition and binding, monomer interaction, filament formation, helicase motifs, ATP binding and hydrolysis, recombination, replication and co-protease activity. Mutational studies have identified residues that are critical to these processes. For example the Gly157 change resulted in a constitutive co-protease form of RecA and results in a lower survival in response to UV treatment, a phenotype itself associated to a low recombination competent form of RecA. The present invention makes use of this information to precisely map regions of RecA to be targeted for compound discovery, *e.g.*, using computational approaches (see below). For example, RecX (also called OraA) is an inhibitor of RecA for both recombinase and co-protease activities (Stohl *et al.*, 2003). RecX appears to inhibit the ATPase activity of RecA. Genetic and biochemical evidence identifies sites of interaction between RecA and LexA, suggesting that amino acids at positions 67, 154-157, 229 and 243 are responsible at least in part for the binding to LexA (VanLook *et al.*, 2003a). Amino acid changes at positions 122-123 and 150-161 dramatically decrease the ability of mutant cells ability to survive in response to UV radiation treatment. A domain in RecA that likely forms part of the co-protease substrate binding site has also

been identified (Nastri *et al.*, 1997). According to the invention this information is used to select portions of RecA for computational screening against small molecule libraries.

[00187] Known inhibitors of RecA that are of use in the invention include a variety of synthetic analogs of ATP and ADP as well as a variety of transition metals, *e.g.*, zinc(II) and copper(II) (Lee, AM and Singleton, F., 2004).

[00188] The RecBCD enzyme is a complex helicase and nuclease (referred to as exonuclease V in *E. coli*) involved in major pathways of homologous recombination and DNA repair; it is also involved in loading RecA onto the DNA end (Singleton *et al.*, 2004). It had been shown that survival after double stranded DNA breakage depends on RecA and RecBCD, and that subsequent events can proceed *via* either of two pathways, one dependent on the RuvABC Holliday junction resolvase and the other on RecG helicase (from Meddows *et al.*, 2004). Reversed forks are reset by the action of RecBC on the DNA double-strand end, and in the absence of RecBC chromosomes are linearized by the Holliday junction resolvase RuvABC.

[00189] The UvrD helicase may be essential for RuvABC-dependent chromosome linearization in *E. coli* polymerase III mutants. UvrD also participates in replication fork reversal in *E. coli* (Flores et al., 2004). UvrD is involved in DNA lesion and mismatch repair. Mutations in *urvD* can cause an increase in the frequency of homologous recombination. UvrD can destroy recombination intermediates made by RecA and can dislodge RecA from ssDNA (Morel *et al.*, 1993; Veaute *et al.*, 2005). The removal of RecA or RecA-made structure by UvrD hence allows replication fork reversal and proper replication restart (Flores *et al.*, 2005; Veaute *et al.*, 2005).

[00190] Fis is a 98 kD homodimeric protein that interacts with DNA, being involved in activities such as bending, looping and condensation of DNA substrates. Fis is involved in DNA recombination, transcription control as either a repressor or activator, in DNA replication, chromosome replication and cell division. Fis as a repressor notably negatively regulates the promoter responsible for its transcription as well as those of the *gyrA* and *gyrB* genes (Walker *et al.*, 1999; Schneider *et al.*, 1999).

[00191] Exonucleases are enzymes that cleave nucleotides sequentially from the free ends of a nucleic acid (DNA or RNA). They have diverse modes of action and are involved in many DNA metabolism processes. This is reflected by the presence of multiple exonuclease encoding genes in the genome of most organisms. The exonuclease VII of *E. coli* degrades single-stranded DNA bidirectionally and processively. It is composed of two subunits encoded by the *xseA* and *xseB* genes (Chase and Richardson, 1977; Chase *et al.*, 1986). It requires a single-stranded DNA terminus to initiate hydrolysis, this unique substrate specificity makes it an ideal agent for various modifications of DNA termini.

[00192] The role of RecN, an ATPase and member of the SMC (structural maintenance of chromosome) protein family, is unclear. A recent study in *Bacillus* proposes that both RecA and RecN constitute or are part of a protein assembly that accepts incoming ssDNA at a cell pole, where it is prepared for recombination with the chromosome (Kidane and Graumannn, 2005). The same authors showed that RecN, like RecA, is an ATP dependent ssDNA binding protein.

[00193] Beta-ketoacyl-acyl carrier protein (ACP) synthase III (KAS III, also called acetoacetyl-ACP synthase), encoded by the *fabH* gene is thought to catalyze the first elongation reaction of type II fatty acid synthesis in bacteria and plant plastids. FabH is believed to conduct the major condensation reaction in the initiation of type II fatty acid biosynthesis in both Gram-positive and Gram-negative bacteria" (Lai and Cronan, 2003). Components of this biosynthetic pathway have been identified as attractive targets for the development of new antibacterial agents. FabH, beta-ketoacyl-ACP synthase III, is a particularly attractive target, since it is central to the initiation of fatty acid biosynthesis and is highly conserved among Gram positive and negative bacteria. Small molecules that inhibit FabH enzymatic activity have the potential to be candidates within a novel class of selective, nontoxic, broad-spectrum antibacterials. Using crystallographic structural information on these highly conserved active sites and structure based drug design principles, a benzoylaminobenzoic acid series of compounds was developed as potent inhibitors of FabH. This inhibitor class demonstrates strong antibacterial activity against Gram-positive and selected Gram-negative organisms. (Nie *et al.*, 2005). However, the utility of inhibitors of FabH (or inhibitors of other enzymes in the fatty acid synthesis pathway) as potentiators of quinolone antibiotics has not heretofore been recognized. The present invention encompasses the use of an inhibitor of bacterial fatty acid synthesis, *e.g.*, an inhibitor of FabH, in combination with a quinolone antibiotic. The inhibitor of fatty acid synthesis may be employed at a concentration or dose below that which would be employed were it to be used as a single agent or may be employed against bacteria that are resistant to the effects of the inhibitor at clinically tolerated doses.

[00194] A set of target genes provided by the present invention is listed in Table 1 (Figure 12). Both the *E. coli* gene and homologs in a variety of other bacterial species are listed. Each *E. coli* gene is identified by its Gene ID (left column). Each polypeptide encoded by a target gene is identified by the GenBank GI number and an accession number of the amino acid sequence of the polypeptide encoded by the gene. Most of the accession numbers are from GenBank (gb); some are from the UniProt database at /www.ebi.uniprot.org (embl). In the case of Corynebacterium, the accession number used is the one assigned by the Sanger Center, which provides the C. diptheriae sequence at www.sanger.ac.uk/Projects/C_diphtheriae/). The contents of the columns in Table 1 are as follows: Column 1 (GENE_ID_ECOLI) presents the Gene ID of an *E. coli* target gene. Column 2 (ECOCYC_ID_ ECOLI) presents the Ecocyc ID of the *E. coli* target gene. Ecocyc (Encyclopedia of Escherichia coli K12 Genes and Metabolism) is a bioinformatics database that describes the genome and the biochemical machinery of *E. coli* K-12 MG1655 and is found at ecocyc.org/background.shtml. Column 3 (K12_GENE) presents the name of the gene in *E. coli*. Column 4 (SUBJECT_ORGANISM) presents the name of the bacterium whose genome was searched to identify a homolog of the *E. coli* gene. Column 5 (SUBJECT_ID) presents the GI number and accession number of a gene that was identified as being homologous to the *E. coli* gene named in the same column. Note that this column provides the GI number and accession numbers of the *E. coli* genes themselves, as found in strain K-12. For example, the GI number and accession number for *E. coli* K-12 AcrB are 1786667 and AAC73564.1, respectively. Column 6 (%_IDENT) presents the % identity of the *E. coli* K-12 polypeptide and its homolog in a second bacterial species. Column 7 (E_VALUE) presents the expectation value for a match between the *E. coli* K-12 polypeptide and its homolog in a second bacterial species. Column 8 (BIT_SCORE) presents the score for a match between the *E. coli* K-12 polypeptide and its homolog in a second bacterial species.

[00195] One of ordinary skill in the art will readily be able to locate the nucleotide sequence of the genes that encode these proteins using publicly available databases. Although the target genes identified herein were identified in a laboratory strain of *E. coli*, the invention encompasses corresponding genes (homologs) in other bacterial species and/or strains. The expression products of the homologs preferably have the same or similar functional activity and will typically display a degree of identity with the *E. coli* polypeptides.

[00196] Given the availability of accession numbers and public databases, it is considered unnecessary to provide sequences for each of the target genes or their encoded polypeptides herein. Furthermore, it will be appreciated that minor differences in sequence of the same gene and, in some cases, the protein, may exist in different bacterial isolates, even within a single species or strain. The invention encompasses all such variants. Figure 13 displays amino acid sequences and accession numbers for the RecA protein from 3 bacterial isolates. The *E. coli* RecA sequence was used as a query sequence to identify the homologs in the other bacteria.

[00197] A variety of methods to identify homologs of target genes are known in the art and include both database searching using either the nucleotide sequence of a target gene or the amino acid sequence of an encoded protein. Since many bacterial genomes have been fully sequenced and are publicly available, the *E. coli* sequence can be used to generate a database query to identify homologs. Either general databases or organism-specific databases can be searched. The complete genome sequence of hundreds of microbes are available at the web site www.ncbi.nlm.nih.gov/genomes/lproks.cgi, which provides links to the sequences. Homologs can be readily identified by one of ordinary skill in the art using programs such as BLASTN, BLASTP, gapped BLAST, etc., which are available on the web site of the NCBI. Default parameters can be used to perform the search. The overall percent identity may be as low as 20%. For purposes of the present invention any bacterial gene that encodes a bacterial polypeptide that is at least 20% identical to a polypeptide encoded by a target gene identified herein (*e.g.*, a target gene identified in Table 1) over the entire length of either polypeptide is considered to also be a target gene, and the polypeptide is considered to be a target polypeptide. However, polypeptides having a lower percent identity are also encompassed. Furthermore, a target gene may display homology only or primarily over one or more portions of the sequence. For example, a homolog may be at least 50%, 60%, 70%, 80%, or 90% identical across one or more portions having a length of about 25, 50, 100, 150, or 200 amino acids. A homolog may be highly conserved or conform to a consensus sequence across a plurality of relatively short motifs (*e.g.*, about 10-30 amino acids). A sequence alignment program may generate a score S and an “expectation value” (E value) for each sequence identified in a comparison. The E value represents the number of different alignments with scores equivalent to or better than S that are expected to occur in a database search by chance. The lower the E value, the more significant the score. In general, if the likelihood that a polypeptide would be identified as a “hit” when a polypeptide encoded by a

target gene identified herein is used to query a database is less than or equal to 10^{-5}, or preferably less than or equal to 10^{-6}, the gene that encodes the polypeptide is considered a target gene, and the polypeptide is a target polypeptide.

Aminoglycoside Potentiator Target Genes

[00198] Using the same method as described in the case of quinolones, the present Applicants have identified a set of target genes by exposing members of a collection of bacterial strains to a sublethal concentration of an aminoglycoside (see Example 34). Bacterial strains that failed to grow and/or survive in the presence of the aminoglycoside were identified. The set of target genes includes *aceE, araJ, aspA, atpA, atpF, atpH, brnQ, cydB, degP, dnaT, fepD, folP, guaB, Hfq, iscS, JW5360, lipA, lpdA, nuoB, nuoJ, nuoL, osmB, pdxH, pnuC, priA, priB, rbfA, recA, rfaD, rpmJ, rpoD, resA, ruvC, sdhC, seqA, setA, sucB, surA, tar, tatE, tauB, trmU, ubiH, ubiX, xerC, yaaU, ycfM, yejO, ygfZ, ygiH, yheL, yheM, yheN, yicG, yjdL, yjeT, yjiO,* and *yjjY.*

[00199] The present invention also provides subset of aminoglycoside potentiator target genes. A subset of target genes is provided that includes *recA, seqA, and atpA, atpF, atpH.*

[00200] The *seqA* gene negatively modulates replication initiation at the E. coli origin, oriC. seqA is also essential for sequestration, which acts at oriC and the dnaA promoter to ensure that replication initiation occurs exactly once per chromosome per cell cycle. Known secA inhibitors include sodium azide, cerulenin, and other compounds such as those described in L.E. Alksne *et al.*,, Antimicrob. Agents Chemother., 2000, 44: 1418-1427.

[00201] An ATP synthase is a general term for an enzyme that can synthesize adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate by utilizing some form of energy. ATP is formed by proton-conducting, membrane-bound ATPase. ATPase is a multicomponent enzyme complex consisting of two main components: F1 and F0. 1 is on the inner surface of the membrane and is the catalytic center; F1 consists of nine polypeptide chain subunits of five different types. F0 is embedded within the membrane and forms the membrane proton channel. In *E. coli*, F0 consists of three subunits (A= atpB, B = atpF, and C = atpE); F1 consists of five subunits (alpha = atpA, beta = atpD, gamma = atpG, delta = atpH, epsilon = atpC).

[00202] Known inhibitors of ATP synthase include mefloquine. Mefloquine (also called mefloquine hydrochloride or Lariam®, Roche Pharmaceuticals) is 4-quinolinemethanol derivative. The exact mechanism of action of mefloquine is unknown, although atpA as been reported to be its target. The antibiotics venturicidin, oligomycin and ossamycin have been investigated as potential inhibitors of the *E. coli* H+ATPase. It was found that venturicidin strongly inhibited ATP-driven proton transport and ATP hydrolysis, while oligomycin weakly inhibited there functions. Inhibition of hte H+ATPase by venturicidin and oligomycin was correlated with inhibition of F0-mediated proton transport.

Peptide Antibiotic Potentiator Target Genes

[00203] Using the same method as described in the case of quinolones and aminoglycosides, the present Applicants have identified a set of target genes by exposing members of a collection of bacterial strains to a sublethal concentration of a cyclic peptide antibiotic (colistin) (see Example 36). Bacterial strains that failed to grow and/or survive in the presence of the cyclic peptide were identified. The set of target genes includes *agaAk, atpA, atpF, atpH, bglF, cysE, cysI, fepC, fepD, frvR, guaA, guaB, hofF, hsdS, iscS, JW4016, JW5075, JW5227, JW5257, JW5360, kdgK, lipA, lysA, malG, mbhA, mdoG, Nei, nmpC, nudH, pdxH, phnB, phnL, phnO, pnuC, potE, pshM, ptsA, rhaT, rpiA, resA, Sbp, speA, sucB, sugE, tdcE, tdcG, tolC, trxA, ubiE, ubiH, ubiX, Xni, ybbY, ycfM, ydeJ, yeeY, yfeT, ygaA, ygfZ, yhdX, yheL, yheM, yiaY, yidK, yihV, yjbN, yjcR, yjcZ, ynjD, yqeC, yqiH,* and *yrfA.*

Potentiator Target Genes Common to Several Classes of Antibiotics

[00204] In certain embodiments, the present invention provides subsets of target genes that are potentiator target genes for more than one class of antibiotics (e.g., 2 or more than 2 classes of antibiotics). In certain embodiments, the present invention provides subsets of target genes that are potentiator target genes for at least two antibiotics (from the same class of antibiotics or from different classes of antibiotics).

[00205] For example, one of such subsets comprises *priA, recA, resA,* and *ruvC,* which have been found to be common to the classes of aminoglycosides and quinolones. Another example of subset of target genes comprises *sucB, ubiH and ubiX, ycfM, ygfZ, yheL and yheM, atpA, atpF, and atpH, iscS, JW5360, guaB, lipA, pdxH, resA* and *pnuC,* which have been found to be common to the classes of aminoglycosides and cyclic peptide antibiotics. Another subset of target genes comprises *ResA* and *RecA.*

Other Methods

[00206] Other methods for identifying target genes in other bacterial species include functional complementation and low stringency nucleic acid hybridization using the *E. coli* gene sequence or a portion thereof (or using the nucleic acid sequence that encodes a homolog in a different bacterial species). Functional complementation could be performed, for example, by transforming a first bacterial strain in which a gene is functionally inactivated with a library of expression vectors containing genes from a second bacterial species or strain in which it is desired to identify a homolog. Transformants are selected and are placed under conditions in which the function of the gene product is required for survival. For example, the transformants may be cultured in the presence of a quinolone antibiotic at a concentration previously determined to be lethal in the absence of a functional gene but sublethal if the gene is functionally active. The expression vector is isolated and the gene it encodes is identified, *e.g.*, sequenced. Low stringency hybridization can be performed using genomic DNA or cDNA from a bacterium in which it is desired to identify a homolog of a target gene using methods described, *e.g.*, in Sambrook, *supra* and well known in the art.

[00207] As noted above, the target gene products used in the methods of the invention include those gene products (*e.g.* RNA or proteins) that are encoded by the target genes identified in Figure 12, Figure 29, Figure 32 and any homologs thereof that exist in other bacterial species or strains. Alternately or additionally, the methods of the invention also use and encompass proteins and polypeptides that represent functionally equivalent gene products. For example, such functionally equivalent gene products include, but are not limited to, natural variants of the polypeptides having an amino acid sequences identified in Figure 13. Functionally equivalent target gene expression products can contain, *e.g.*, deletions, additions or substitutions of amino acid residues within the amino acid sequences encoded by the target gene sequences described above, but which result in a silent change, thus producing a functionally equivalent target gene product. Alternately or additionally, amino acid substitutions can be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues involved. For example, nonpolar (*i.e.*, hydrophobic) amino acid residues can include alanine (Ala or A), leucine (Leu or L), isoleucine (Ile or I), valine (Val or V), proline (Pro or P), phenylalanine (Phe or F), tryptophan (Trp or W) and methionine (Met or M); polar neutral amino acid residues can include glycine (Gly or G), serine (Ser or S), threonine (Thr or T), cysteine (Cys

or C), tyrosine (Tyr or Y), asparagine (Asn or N) and glutamine (Gin or Q); positively charged (i.e., basic) amino acid residues can include arginine (Arg or R), lysine (Lys or K) and histidine (His or H); and negatively charged (ie., acidic) amino acid residues can include aspartic acid (Asp or D) and glutamic acid (Glu or E).

[00208] A functionally equivalent polypeptide, as used herein, refers to a polypeptide that exhibits a substantially similar *in vivo* activity as the polypeptide to which it is functionally equivalent. When used in an assay such as those described below, the term "functionally equivalent" encompasses peptides or polypeptides that are capable of interacting with other cellular or extracellular molecules in a manner substantially similar to the way in which the corresponding portion of the target gene product would interact with such other molecules. Functionally equivalent target gene products are typically about the same size or identical in size to the polypeptide with which they are functionally equivalent and will typically have similar physicochemical properties such as pI. The invention explicitly includes any polypeptide having at least 80% identity, *e.g.*, 90% identity, or more to any of the polypeptides identified by accession number in Figure 12, Figure 29, Figure 32 and nucleic acids encoding such polypeptides, whether or not such polypeptides or nucleic acids are found in nature.

IV. Screening Assays to Identify Agents that Potentiate Antibiotic Activity

[00209] The invention provides screens that may be used to identify agents that modulate the expression of any antibiotic potentiator target gene or that modulate the activity of an expression product of an antibiotic potentiator target gene. Certain preferred screens identify agents that inhibit expression of the gene or inhibit one or more activities of a polypeptide encoded by the gene. "Inhibit", when used in connection with an agent that inhibits an activity of a protein, *e.g.*, enzymatic activity, means to directly or indirectly reduce the activity of the protein. Preferably an agent that inhibits a protein causes a decrease in maximum activity of at least 50% at a concentration of approximately 500 μM or less, preferably at a concentration of 200 μM or less, more preferably at a concentration of 100 μM or less. "Inhibit or interfere with" can encompass mechanisms such as causing a reduction in the amount of a cofactor or substrate, etc.,

[00210] The screens may advantageously be performed using a high throughput format. High throughput screens typically make use of microwell plates (*e.g.*, 96-well, 384-well, 1596-well, etc.) and may employ robotics for various steps such as liquid handling,

compound dispensing, plate manipulation, etc. For example cells, or populations of cells, are dispensed into individual vessels, *e.g.*, wells in a multiwell plate. One or more test agents is added to each well, and information about one or more parameters, *e.g.*, cell growth, is gathered. Plate readers can be used to detect signals such as optical density, colorimetric or fluorescent readouts, etc. The readout may be indicative of growth of the cells (*e.g.*, cell density). However, a variety of other indicators may be assessed.

[00211] In general, the assays will make use of a biological system, which may be a cell-free or cell based system. Cell-free assays refer to assays that are performed using a biological system that does not include intact cells but includes one or more biological macromolecules, *e.g.*, protein(s) and/or nucleic acid(s). Cell-based assays employ intact cells. The cells may be maintained in liquid culture or on a solid or semi-solid culture medium. The biological system may comprise purified or partially purified nucleic acids and/or proteins, cell lysates, cellular fractions such as cell membrane preparations, cells, etc. The biological macromolecules may be chemically synthesized, produced by recombinant DNA technology, obtained from natural sources (*e.g.*, from cells, etc.)

[00212] Briefly, a "recombinant protein" refers to a protein which is produced by recombinant DNA techniques, wherein generally, DNA encoding a polypeptide is inserted into a suitable expression vector which is in turn introduced into a host cell to produce the recombinant protein. Vectors may be introduced into cells using any of a variety of suitable methods. Methods for introducing nucleic acids into cells are well known in the art. One of ordinary skill will be able to select appropriate cells for expression and an appropriate method (*e.g.*, calcium phosphate or lipid-mediated transfection, electroporation, bacterial or fungal transformation, etc.) for introducing a nucleic acid into the cells, taking into consideration the cell type, etc. Suitable host cells for producing recombinant proteins include bacteria, yeast, insect cells, mammalian cells, etc. The biological macromolecules typically include an expression product of a target gene or a functional equivalent thereof. Alternately the biological macromolecule can be a variant of a polypeptide encoded by a target gene. Certain variants are specifically engineered, *e.g.*, by point mutation, deletion, truncation, etc., to alter a biological activity of a protein. For example, constitutively active forms can be produced. Inactive forms can be produced, which may act antagonistically to a naturally occurring protein, *e.g.*, by competing for binding to another component in a pathway. In certain embodiments an active fragment or domain of a protein is used. Typically the active fragment or domain comprises at least 50 amino acids of the complete polypeptide. Certain fragments retain at least one biological activity of the complete polypeptide, *e.g.*, enzymatic activity, inhibitory activity, binding activity to a substrate or to an interacting polypeptide, etc. Additional preferred biological macromolecules that may be included in a biological assay system include substrates for a target polypeptide.

[00213] The biological macromolecules may be modified for use in the assays. For example, a polypeptide may incorporate a heterologous sequence, *e.g.*, an epitope tag such as a GST, Myc, HA, FLAG™, maltose-binding domain, 6X-His or other metal binding moiety, etc. Fusion proteins in which a detectable polypeptide is fused to a polypeptide encoded by a target gene are also of use. Detectable polypeptides include fluorescent proteins such as green fluorescent protein (GFP) and variants thereof. A number of enhanced versions of GFP (eGFP) have been derived by making alterations such as conservative substitutions in the GFP coding sequence. Other readily detectable markers that produce a fluorescent signal include red, blue, yellow, cyan, and sapphire fluorescent proteins, reef coral fluorescent protein, etc. A wide variety of such markers is available commercially, *e.g.*, from BD Biosciences (Clontech). Enzymatic markers include, *e.g.*, β-galactosidase, chloramphenical acetyltransfersase, alkaline phosphatase, horseradish peroxidase, etc. Additional readily detectable markers preferred in certain embodiments of the invention include luciferase derived from the firefly (*Photinus pyralis*) or the sea pansy (*Renilla reniformis*). Methods for detecting expression of any of these markers are well known in the art. For example, expression of β-galactosidase can be quantified using a biochemical colorimetric detection assay (Miller, 1972). Other markers are readily detectable based on their fluorescence or luminescence or based on their ability to produce a fluorescent or luminescent signal upon cleaving a substrate.

[00214] Other reporters include enzymes that cleave a substrate, wherein the substrate has a fluorescent moiety and a fluorescence quencher attached thereto. Cleavage separates the fluorescent moiety from the quencher, resulting in a detectable increase in the fluorescent signal. The biological system may comprise one or more antibodies, agonists, antagonists, etc., for any of the afore-mentioned proteins, inhibitors of transcription, inhibitors of translation, etc.

[00215] Reporter constructs are also of use in certain cell-based assays that are designed to identify agents that modulate expression of a target gene. For example, a reporter construct can include the promoter of a target gene, operably linked to a nucleic acid

sequence that encodes a detectable marker. An expression vector containing the reporter construct is used to transform cells. Expression results in a detectable signal. Compounds are applied to the cells (in individual vessels or wells), and expression is detected. Compounds whose presence results in altered expression are identified. Optionally the screen can be performed in the presence of an antibiotic, *e.g.*, a quinolone.

[00216] In one embodiment the invention provides an assay system comprising RecA protein, a reporter construct containing a *recA* regulatory element (*e.g.*, the *recA* promoter or a functional portion thereof) operably linked to a nucleic acid sequence that encodes a detectable marker, and a test compound. Typically the assay is a cell-based assay, and the reporter construct is introduced into cells that express RecA. However, cell-free transcription/translation systems could also be used. Transcription of the nucleic acid sequence is responsive to RecA, such that higher transcription (and then translation) occurs in the presence of active RecA. Thus test agents that inhibit RecA result in decreased fluorescence, allowing the identification of RecA inhibitors. The invention further provides a method of identifying a compound comprising providing an assay system comprising RecA protein, a reporter construct containing a *recA* regulatory element (*e.g.*, the *recA* promoter or a functional portion thereof) operably linked to a nucleic acid sequence that encodes a detectable marker, and a test compound and determining whether the compound inhibits expression of the detectable marker. Optionally the assay is a cell-based assay, in which bacterial cells express RecA and contain the RecA responsive reporter construct. Optionally the method includes a step of subjecting the cells to a condition that induces RecA synthesis and/or activation.

[00217] Reporter constructs are also of use to identify compounds that inhibit the activity of a target gene that encodes a transcription factor or subunit thereof. A reporter construct containing a coding sequence for a detectable marker can be fused to a regulatory region (*e.g.*, a promoter) of gene known to be regulated by the transcription factor. Activity of the transcription factor is assessed based on the detectable signal generated by the marker. The ability of a test compound to inhibit transcription is assessed. Note that the precise boundaries of a regulatory region need not be defined in order for it to be usable in a reporter construct, provided that it contains sufficient sequences to render transcription of the reporter responsive to the presence, absence, or amount of a particular transcription factor, repressor, inducer, or condition of interest, etc.

[00218] Binding assays are of use to identify agents that bind directly to target molecules such as those encoded by the target genes of the invention. Screening for compounds that bind to a polypeptide of interest has traditionally been performed using a variety of *in vitro* techniques such as radioligand binding assays, photocrosslinking, and affinity chromatography. These assays are based on the principles of a competitive binding assay in which a radiolabeled ligand competes with an unlabeled ligand for binding to a target polypeptide. The ability of a test agent to compete with a known ligand is assessed by measuring the binding of the labeled known ligand in the presence of a test agent. A decrease in the binding of the labeled known ligand indicates that the test agent binds to the target molecule. Whether the test agent inhibits or activates the target is then determined using other methods, *e.g.*, enzymatic assays, cell-based assays, etc. When performing such assays, any known ligand or substrate for the various target polypeptide can be used. For example, ATP or an analog thereof could be used to identify compounds that bind to an ATPase such as RecA or any of the helicases or helicase-like target polypeptides described herein. Methods for performing radioligand binding assays are well known in the art, and a number of kits are commercially available. See, *e.g.*, Rahman, A., *et al.*, *Bioassay Techniques for Drug Development, supra.* Examples of commercially available kits include the FlashPlate™ system (DuPont-NEN), the "Scintillation Proximity™" assay (Amersham), ScintiStrip™ plates (Wallac), etc. Ligands labeled with nonradioactive detectable moieties, *e.g.*, fluorescent moieties, may alternatively be used.

[00219] Methods based on fluorescence polarization and surface plasmon resonance are increasingly employed to detect molecular interactions such as that between a polypeptide and a potential modulator. The phenomenon of surface plasmon resonance is used in Biacore systems (available from Biacore International AB, Neuchâtel, Switzerland). Such systems can be used to detect interactions between a protein of interest and a test agent. As described by Biacore, detection using surface plasmon resonance sensors works as follows: As molecules are immobilized on a sensor surface the refractive index at the interface between the surface and a solution flowing over the surface changes, altering the angle at which reduced-intensity polarized light is reflected from a supporting glass plane. The change in angle, caused by binding or dissociation of molecules from the sensor surface, is proportional to the mass of bound material and is recorded in a sensorgram. When sample is passed over the sensor surface, the sensorgram shows an increasing response as molecules

interact. The response remains constant if the interaction reaches equilibrium. When sample is replaced by buffer, the response decreases as the interaction partners dissociate.

[00220] Many of the polypeptides encoded by target genes are part of multisubunit proteins or macromolecular complexes. Agents that disrupt interactions between a target polypeptide and one or more of the polypeptides (or nucleic acids) to which it binds in the bacterial cell effectively inhibit the activity of the polypeptide and are of use as antibiotic potentiating agents. Formation of complexes between a protein of interest and one or more other proteins may be detected using a number of methods well known in the art and can be performed in either cell-free or cell-based systems. Methods for detection include immunological methods, chromatographic methods, etc. Frequently it will be desirable to detectably label the polypeptide of interest, *e.g.*, with a fluorescent or radioactive label, and/or to epitope tag the protein of interest. Compounds are then screened to determine whether they prevent complex formation and/or disrupt complexes that have already formed.

[00221] In many of the assays, the protein of interest or a potential binding protein is immobilized, *e.g.*, in a vessel such as a microtiter plate or microfuge tube, to a chromatographic matrix, etc. Immobilization may be accomplished using crosslinking agents or antibodies or by biotinylating the protein and utilizing a vessel to which avidin is attached. In some embodiments a fusion protein comprising a protein of interest and heterologous sequence comprising a binding domain (*e.g.*, GST, 6X-His, maltose binding domain, etc.) is generated. After immobilizing the protein, the protein is contacted with a potential interacting protein (either partially or fully purified, or in a cell lysate, etc.). After a period of incubation, a wash is performed to remove unbound material. Complex formation can be detected, *e.g.*, using an antibody that binds to the potential interacting protein. The invention contemplates use of antibodies that bind to a target polypeptide for use in the assays described herein. Methods for producing monoclonal or polyclonal antibodies are well known in the art. It is noted that the term “antibody” as used herein encompasses antibody fragments, single chain antibodies, other proteins that contain an antigen-binding domain, etc. Alternately, the interacting protein may be detectably labeled (*e.g.*, enzymatically, fluorescently, etc). The ability of an agent to promote or inhibit complex formation is assessed by allowing complex formation to occur in the presence of the agent (or by adding the agent following complex formation) and comparing the extent of complex formation with that occurring in the absence of the agent. Proteins can also be subjected to various procedures that involve separation based on size. Complex formation results in a

detectable increase in size. Methods for detecting the increase in size include chromatography, gel electrophoresis, etc.

[00222] A widely used method for detecting protein-protein interactions is the two-hybrid approach, which is described in U.S. Pat. Nos. 5,283,173; 5,468,614; and 5,667,973. Briefly, the method is based on reconstituting a functional transcriptional activator protein from two separate fusion proteins in a biological system, preferably in living cells in culture, although *in vitro* biological systems other than intact cells, (optionally containing cellular constituents) could also be employed. This reconstitution makes use of chimeric genes which express fusion proteins. At least one of the fusion proteins contains a protein of interest or a portion thereof. The other fusion protein contains a known or potential interacting protein. Each fusion protein also contains a domain of a transcriptional activator, *e.g.*, one fusion protein contains a DNA binding domain and the other fusion protein contains a transactivation domain. Interaction between the fusion proteins reconstitutes a transcriptional activator, leading to expression of a reporter construct that contains a binding site for the transcriptional activator fused to a sequence that encodes a reporter protein. A variety of suitable reporter proteins are known in the art. Expression of the reporter construct is detected and provides an indication that interaction has occurred. The chimeric gene encoding the fusion protein containing the interacting protein can then be isolated, allowing identification of the interacting protein. Numerous variants and improvements on this method have been made since its initial description and can be employed in the present invention. For example, transcriptional repression domains could also be used, wherein the readout would be reduced expression of the reporter construct if an interaction occurs.

[00223] The two-hybrid system can be used to identify agents, *e.g.*, small molecules, that disrupt interaction of two known polypeptides. In this approach, a transcriptional activator is reconstituted as described above using first and second fusion proteins, each of which contains a domain of a transcriptional activator and one of the known interacting polypeptides (or a portion thereof). Reconstitution of the transcriptional activator results in expression of a reporter construct, which can be detected. A test agent is added to the biochemical system. If the test agent disrupts the interaction, a decrease in expression of the reporter construct will be detected.

[00224] Three hybrid screening assays are also of use in the present invention. These assays are useful for screening chemical libraries, *e.g.*, libraries of small molecules, to identify agents that can bind to particular targets of interest. Methods and reagents for

performing three hybrid screening assays are described in U.S. Pat. No. 5,928,868 and in Licitra et al., *Proc. Natl. Acad. Sci. USA* 93: 12817-21 (1996). Briefly, the three hybrid assay involves the formation of a complex between a hybrid ligand and two hybrid proteins in which a portion of a component of the three hybrid complex may be unknown. The unknown component can be either a small molecule that forms part of the hybrid ligand or forms part of one of the hybrid proteins. The three hybrid assay is based on a similar concept to the two hybrid assay described above, *i.e.*, formation of a complex (in this case a three component complex) triggers the expression of a reporter gene. Expression of the reporter gene is detected using a suitable technique and indicates interaction of the members of the complex. The unknown component is then identified.

[00225] In the context of the present invention the three hybrid assay can be used for any one or more of the following purposes: (i) determining the identity of a small molecule capable of direct binding to a known target polypeptide where the identified small molecule may be suitable as a modulator of the activity of the target polypeptide or (ii) determining the identity of a small molecule capable of binding competitively to a target polypeptide in the presence of a hybrid molecule so as to inhibit the binding between the target and a second small molecule that forms part of the hybrid molecule (*e.g.*, the second small molecule may be a known ligand for the target molecule.

[00226] Cell-free assays based on the enzymatic activity of various target polypeptides of the present invention are also of use. For example, compounds can be screened to identify those that inhibit ATPase activity, helicase activity, exonuclease activity, etc. In one embodiment an assay system containing an isolated polypeptide having ATPase activity (*e.g.*, RecA) is provided. The assay system also contains ATP and reagents suitable for detecting ATP or for detecting products formed by hydrolysis of ATP. A suitable assay is described in the Examples. In that assay, ATP remaining after a period of incubation of an ATPase (*e.g.*, RecA) in the presence of ATP and, optionally, a test agent (potential inhibitor) is assessed. ATP can be detected using a variety of methods known in the art. In another embodiment a purified polypeptide (*e.g.*, RecA or any helicase or helicase-like polypeptide) is incubated with 1 μg of DNA (Sigma D3287 or D8681) in 20 mM HEPES (pH 7.6; 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid), 5 mM $MgCl_2$, 0.2-5 mM ATP, 100 μg of BSA (bovine serum albumin) per ml, 10% glycerol, 1 mM DTT (DL-dithiothreitol) and, optionally, a test agent (potential inhibitor) in the appropriate concentration at 37 degrees C for 60 min. The liberated inorganic phosphate is determined by colorimetry as described in

"An improved assay for nanomole amounts of inorganic phosphate", 1979, P. A. Lanzetta, I. J. Alvarez, P. S. Reinach and O. A. Candia, Analytical Biochemistry, 100:95-97. The inhibition of the DNA-dependent ATPase activity may be calculated from the change in the absorption in the presence and absence of inhibitor. These ATPase assays are only two of the numerous options which are available to the skilled person and are detailed, for example, in "High-Throughput Screening Assay for Helicase Enzymes", 1998, M. Sivaraja, H. Giordano and M. G. Peterson, Analytical Biochemistry, 265, 22-27; Berger MD, *et al.*, Biochem Biophys Res Commun. 2001;286:1195–203, and Morrical SW, *et al.* Biochemistry. 1986;25:1482–94. In certain embodiments of the invention an agent identified using the inventive method inhibits ATPase activity of RecA but not of the related mammalian protein Rad51 (reviewed in Kawabata *et al.*, 2005).

[00227] In another embodiment an assay to measure exonuclease activity is used. Many methods for detecting and optionally measuring exonuclease activity are known in the art. The assay system typically includes an isolated exonuclease polypeptide, a nucleic acid substrate, and any appropriate cofactors, salts, buffers, etc. The specific assay and nucleic acid substrate selected may depend at least in part on whether the exonuclease degrades double or single-stranded nucleic acid, whether it is specific for RNA or DNA, whether it degrades in a 5' to 3' or 3' to 5' direction, etc. For example, exonuclease activity can be measured using nucleic acids that incorporate detectably labeled nucleotides (*e.g.*, radiolabeled, fluorescently labeled, etc). Alternately, a nucleic acid can incorporate a fluorescent reporter dye and a fluorescent quencher dye. While both the reporter and the quencher are in proximity with one another, fluorescence is quenched. However, when the nucleic acid is degraded, the reporter and quencher are no longer in proximity, and fluorescence is detected. This is assay, based on a similar principle to the widely used Taqman® assay for detecting PCR amplification, is highly suitable for high throughput format. In another embodiment, an exonuclease is incubated with a nucleic acid substrate for a period of time. Following incubation, gel electrophoresis is performed and the integrity of the nucleic acid is assessed. The above assays are performed in the presence of a test agent and the ability of the test agent to inhibit cleavage is assessed.

[00228] In another embodiment, an *in vitro* recombination assay using Fis and a Int proteins in conjunction is employed. Suboptimal Int protein activity can be enhanced by adding increasing amount of Fis in the reaction. Compounds are tested for their ability to

prevent recombination when present in an assay system containing Fis, Int, and an optionally labeled nucleic acid substrate for recombination. For example, a labeled DNA substrate can be used, and substrate and products can be quantified, *e.g.*, by separation using gel electrophoresis (Esposito and Gerard, 2003). The substrate can be, *e.g.*, fluorescently labeled, radioactively labeled, etc. The invention therefore encompasses an assay system comprising Fis, Int, a nucleic acid substrate, and a test compound. The invention further encompasses a method of identifying a compound comprising providing an assay system containing Fis, Int, a nucleic acid substrate such as labeled DNA, and a test compound and assessing the ability of the compound to inhibit recombination. A compound that inhibits recombination is identified as an inhibitor of Fis and is a candidate quinolone potentiating agent.

[00229] In another embodiment the ability of DNA binding protein such as Fis or DksA to bind DNA can be measured using gel retardation assays (Esposito and Gerard, 2003; Choi *et al.*, 2005). The effect of a test compound on such binding is determined. A compound whose presence inhibits binding of the DNA binding protein to DNA is identified as an inhibitor of binding and as a candidate quinolone potentiating agent.

[00230] In another embodiment a reporter assay is used to identify compounds that inhibit Fis or DksA. The invention therefore encompasses an assay system comprising Fis or DksA and a reporter construct operatively linked to a transcriptional regulatory sequence (*e.g.*, a promoter) of a gene whose transcription is regulated at least in part by Fis or DksA (*e.g.*, an rRNA gene), and a test compound. The invention further encompasses a method of identifying a compound comprising providing an assay system containing Fis or DksA, a reporter construct containing a regulatory region of a gene whose transcription is regulated at least in part by Fis or DksA, and a test compound and assessing the ability of the compound to inhibit expression of the reporter. A compound that inhibits expression is identified as an inhibitor of Fis or DksA, respectively, and is a candidate quinolone potentiating agent. The invention encompasses such an assay system and method of compound identification for any transcription factor or subunit thereof identified as being encoded by an antibiotic potentiator target gene.

[00231] Cell-based assays to identify agents that inhibit a target gene of interest can also involve exposing cells that express different amounts of a target gene to a sublethal concentration of an antibiotic. The cells are contacted with a test agent. An agent that

inhibits growth or survival of the cells that express lower levels of the target gene to a greater extent than it inhibits growth or survival of cells that express a higher amount of the target gene is a potential inhibitor of the polypeptide encoded by the target gene or a potential inhibitor of expression of the target gene. In an exemplary embodiment the ability of wild type cells to grow or survive in the presence of a sublethal concentration of a quinolone and a test agent is compared with the ability of cells that overexpress the target gene to survive in the presence of the same concentration of the quinolone and the test agent. If the test agent has a greater inhibitory effect on the wild type cells, the test agent is identified as a potential inhibitor of the target gene or an expression product thereof and, accordingly, as a candidate quinolone potentiating agent. Of course any agent identified in any of the inventive cell-free or cell-based assays may be subjected to additional testing either in culture or in animal models of bacterial infection.

[00232] Compounds suitable for screening according to the inventive methods include small molecules, natural products, peptides, nucleic acids, etc. Sources for compounds include natural product extracts, collections of synthetic compounds, and compound libraries generated by combinatorial chemistry. Libraries of compounds are well known in the art. One representative example is known as DIVERSet^{TM}, available from ChemBridge Corporation, 16981 Via Tazon, Suite G, San Diego, CA 92127. DIVERSet^{TM} contains between 10,000 and 50,000 drug-like, hand-synthesized small molecules. These compounds are pre-selected to form a "universal" library that covers the maximum pharmacophore diversity with the minimum number of compounds and is suitable for either high throughput or lower throughput screening. For descriptions of additional libraries, see, for example, Tan, *et al.*, “Stereoselective Synthesis of Over Two Million Compounds Having Structural Features Both Reminiscent of Natural Products and Compatible with Miniaturized Cell Based Assays”, Am. Chem Soc.120, 8565 8566, 1998; Floyd CD, Leblanc C, Whittaker M, Prog Med Chem 36:91-168, 1999. Numerous libraries are commercially available, e.g., from AnalytiCon USA Inc., P.O. Box 5926, Kingwood, TX 77325; 3-Dimensional Pharmaceuticals, Inc., 665 Stockton Drive, Suite 104, Exton, PA 19341-1151; Tripos, Inc., 1699 Hanley Rd., St. Louis, MO, 63144-2913, etc.

[00233] Methods of synthesizing and encoding combinatorial libraries are known in the art. Libraries of compounds are screened to identify compounds that function as modulators, *e.g.*, inhibitors of the target gene product. For example, libraries of small molecules may be generated using methods of combinatorial library synthesis well known in the art. Library

compounds may be provided in solution or may be attached to a solid support such as a bead. In certain embodiments of the invention the compounds to be tested are synthesized to contain a common core structure. The core structure may be one that characterizes a compound shown to display activity against a particular gene product (*e.g.*, using a cell-free assay) and/or predicted to display activity based on computational approaches. Once a library of compounds is screened, subsequent libraries may be generated using those chemical building blocks that possess the features shown in the first round of screen to have activity against the target gene product. Using this approach, subsequent iterations of candidate compounds will possess an increasing number of those structural and functional features required to inhibit or otherwise modulate the function of the target, until a group of compounds with high activity and, optionally, specificity for the target can be found. These compounds can then be further tested for their safety and efficacy for therapeutic use.

[00234] Computational methods of use to identify an agent that modulates activity of a polypeptide encoded by a target gene are also provided by the present invention. Molecular modeling can be used to identify a pharmacophore for a particular target site, *e.g.*, the minimum functionality or set of desirable features that a molecule should have to possess activity at that target site. Such modeling can be based, for example, on a predicted or known structure for the target (*e.g.*, a two-dimensional or three-dimensional structure). Software programs for identifying such potential lead compounds are known in the art, and once a compound exhibiting activity is identified, standard methods may be employed to refine the structure and thereby identify more effective compounds. Binding interactions between test agents and a site on a target molecule (*e.g.*, a polypeptide encoded by an antibiotic potentiator target gene of this invention) can be determined by molecular modeling programs that are known to those of ordinary skill in the art. These molecular modeling programs include QUANTA (Accelrys Inc., San Diego, CA) and the SYBYL suiite of computational informatics software (Tripos Associates, Inc., St. Louis, Mo.)

[00235] Structures of a number of the targets described herein are known in the art. For example, the structure of RecA, in some cases bound to a substrate and/or to DNA has been reported. The structures have been established for bacteria such as *E. coli* (Rossbach et al., 2005; Story and Steitz, 1992; Story, Weber and Steitz, 1992; VanLoock et al., 2003b; Xing and Bell, 2004; Yu and Egelman, 1997); *Mycobacterium* sp. (Datta et al., 2000, Datta et al., 2003), *Proteus mirabilis* (Weber and Steitz, 1986) and in *Archea* (Ariza et al., 2005; Wu et al., 2004). The human RecA homolog Rad51 structure has been also determined (Conway et al., 2004; Wu et al., 2005); this structure can allow filtering for compounds that specifically dock with bacterial RecA and not with Rad51 (Aihara et al., 1999; Shinohara and Ogawa, 1999; Yu et al., 2001).

[00236] Software programs for performing computer-based screening are now in common use. Examples include DOCK, FlexX™, FRED, GOLD, and ICM. Although the approach can vary, these programs typically involve two major phases: searching and scoring. During the first phase, the program automatically generates a set of candidate complexes of two molecules (test compound and target molecule) and determines the energy of interaction of the candidate complexes. The scoring phase assigns scores to the candidate complexes and selects a structure that displays favorable interactions based at least in part on the energy. To perform virtual screening, this process is repeated with a large number of test compounds to identify those that display the most favorable interactions with the target. A database of commercially available compounds has been constructed for such use: the ZINC database consists of over 700,000 molecules, each with 3-dimensional structure, which are readily and freely available for this purpose (Irwin and Shoichet, 2005). Other databases are known in the art. Genetic and biochemical data may be employed to focus the *in silico* screen. For example, structures for screening can be selected based on known ligands and/or chemical information can be used to actively guide the orientation of the ligand into the binding site. See, *e.g.*, Fradera, X. and Mestres, J., 2004. Computation-based approaches to drug discovery have been extensively reviewed. See, e.g., Schneidman-Duhovny, D., et al., 2004; Brooijmans, N. and Kuntz, I.D., 2003; Alvarez, JC, 2004. See also, Tollenaere, J., et al. (eds.) *Computational Medicinal Chemistry and Drug Discovery*, New York: Marcel Dekker, 2004.

[00237] Compounds which virtually bind to a target protein *in silico* can be evaluated to determine whether they are actual inhibitors of activities of the target protein either in cell-free or cell-based assays such as those described above. Recent successes confirm the utility of the virtual screening approach, which is now a common technique in early stage discovery and has yielded considerable success. Drugs such as Gleevec (oncology, Novartis), Relenza (influenza virus, GlaxoSmithKkline), Tamilflu (influenza virus, Roche) and Agenerase (HIV infection, VertexPharma) are examples that show the successful contributions of rational design (Ng, 2004, Shoichet, 2004).

[00238] In addition, known ligands or ligands identified can be modified, and the effects of the modified compounds on protein activity and/or antibiotic potentiation can be assessed.

Thus any of the above screening methods may be performed using a modified version of an agent identified using one of the inventive screens or otherwise identified as having activity towards one of the targets mentioned herein.

[00239] The invention also provides a computer-readable medium on which are stored results of a screen to identify an agent that potentiates activity of an antibiotic, *e.g.*, a quinolone. The results may be stored in a database and can include any screening protocols, results obtained from the screen or from additional screens, and/or protocols of or results obtained from tests performed on compounds identified in the screen (*e.g.*, tests in animal models of infection).

[00240] The invention further provides a method of conducting a business to identify a therapeutic agent, *i.e.*, a compound that potentiates an antibiotic. The method involves performing any of the screens described herein, optionally to identify a compound that potentiates the activity of a marketed antibiotic agent, a non-marketed agent known to have antibiotic activity, an agent not known to have antibiotic activity, etc. The screens can be performed on a contract basis, *e.g.*, as a service, in which a customer requests that a screen be performed to identify a compound that potentiates activity of an antibiotic agent suggested by or provided by the customer. In certain embodiments of this method the agent for which a potentiating agent is desired is a quinolone antibiotic, an aminoglycoside antibiotic, or a lactam antibiotic.

V. Antibiotic Potentiation and Resistance Suppression via RecA Inhibition

[00241] As already mentioned above, *recA* was found to be a common antibiotic potentiator target gene of quinolones, and aminoglycosides. Accordingly, the present invention provides RecA inhibitors that potentiate the activity of other antibiotic agents. When RecA inhibitors are administered in combination with one or more such antibiotic agents whose activity they potentiate, the antibiotic agent(s) may often be utilized at a lower dose, and/or less frequent dosing regimen than their conventional dose and/or schedule. In some embodiments, addition of one or more RecA inhibitors to an antibiotic therapy regimen significantly reduces the survival of bacteria at conventional therapeutic antibiotic agent dosing. In some embodiments, inventive RecA inhibitors reduce the incidence of resistance developed toward one or more antibiotic agents. In some embodiments, RecA inhibitors retard resistance developed toward one or more antibiotic agents.

[00242] In general, agents that inhibit one or more activities of RecA, and/or that inhibit RecA expression levels, may be useful in accordance with the present invention. Exemplary RecA activities that may be inhibited include, but are not limited to, DNA binding, monomer interaction, helicase activity, filament formation, ATP binding and/or hydrolysis, co-protease activity (*e.g.*, toward LexA and/or UmuD), recombinase activity, replication function, and combinations thereof. In some embodiments, inventive RecA inhibitors inhibit one or more such activities with an IC_{50} below about 100 μg/ml, 50 μg/ml, 15 μg/ml; 10 μg/ml; 5 μg/ml, 3 μg/ml, or 1 μg/ml. According to the present invention, desirable RecA ATPase inhibitors may even have an IC_{50} well below 1 μg/ml, or even below 500 ng/ml, 100 ng/ml, 50 ng/ml, 30 ng/ml,. 25 ng/ml, 20 ng/ml, 15 ng/ml, 10 ng/ml, 5 ng/ml, 1 ng/ml, or less.

[00243] In some embodiments of the present invention, RecA inhibitors are broad spectrum agents in that they inhibit RecA (or the relevant RecA homolog) from more than one different microbial source. In other embodiments, RecA inhibitors have a narrow spectrum activity in that they inhibit one or more activities of RecA (or its relevant homolog) from a specific family of organisms or from a specific organism. In certain preferred embodiments, RecA inhibitors inhibit one or more activities of RecA (or its relevant homolog) from a disease-causing organism (in particular an organism that causes disease in a mammal, *e.g.*, a human). In some embodiments, however, the RecA inhibitors (which may be broad spectrum with regard to microbes) do not inhibit RecA (or the relevant RecA homolog) from one or more higher organisms (*e.g.*, mammals, humans). For example, in some embodiments, RecA inhibitors do not inhibit RAD51.

[00244] In some embodiments, the present invention provides RecA inhibitors that inhibit the RecA ATPase activity. For example, the present invention demonstrates that a variety of compounds inhibit RecA ATPase activity in an *in vitro* luciferase assay (see, for example, Example 24). The present invention specifically provides the compounds depicted in Figure 14 as RecA inhibitors with the indicated IC_{50}s in the *in vitro* luciferase assay.

[00245] In some embodiments of the present invention, RecA inhibitors that inhibit the RecA ATPase activity do not inhibit certain other cellular ATPases.

[00246] In some embodiments, the present invention provides RecA inhibitors that bind directly to RecA. In some embodiments, RecA inhibitors bind to the RecA ATP binding site. However, in some embodiments, inventive RecA inhibitors do not bind to the RecA ATP binding site (even though they may inhibit the RecA ATPase activity). In certain

embodiments, inventive RecA inhibitors bind to two or more different sites on the RecA protein. For example, Example 31 proposes two potential new binding sites for RecA inhibitors, including those that inhibit ATPase activity.

[00247] In some embodiments, inventive RecA inhibitors that bind directly to RecA bind to a site comprised of amino acid residues including R85, F270, Y271, K310, and/or R324 (see, for example, Example 31). According to the present invention, this site can be found on the outer surface of RecA, as positioned in a filament. According to the present invention, hinokiflavone may bind to this RecA site. Further according to the present invention, compounds that compete with hinokiflavone for binding to RecA may be desirable RecA inhibitors. As used herein, the term "competes with hinokiflavone for binding to RecA", when used to characterize a compound, refers to a compound that has binding properties to RecA similar to (*i.e.*, similar binding site(s) than) hinokiflavone).

[00248] In certain embodiments of the present invention, RecA inhibitors potentiate the activity of one or more antibiotic agents. For example, the present Applicants have found that hinokiflavone potentiates activity of a quinolone antibiotic (ciprofloxacin). Moreover, this potentiation is only observed against cells that express RecA.

[00249] In some embodiments of the present invention, RecA inhibitors reduce the incidence of resistance that develops to one or more antibiotic agents. In some embodiments of the present invention, RecA inhibitors retard the emergence of resistance that develops to one or more antibiotic agents. For example, hinokiflavone was found to reduce the incidence of resistance to ciprofloxacin. The generality of hinokiflavone's mechanism of action (inhibiting RecA) suggests that this compound should also suppress or retard resistance to and/or potentiate activity of other antibiotic agents.

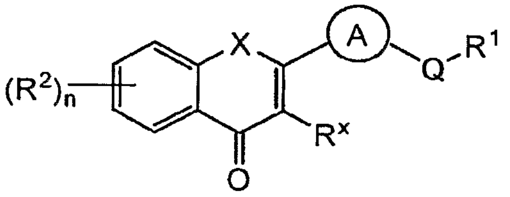
[00250] In certain embodiments of the present invention, RecA inhibitors show intrinsic antibiotic activity, even in the absence of any other antibiotic agent. However, antibiotic activity is not necessary for antibiotic potentiating and/or antibiotic resistance suppression.

[00251] In other embodiments, RecA inhibitors can actually protect cells from death. Without wishing to be bound by any particular theory, it is noted that one possible explanation for the present findings is that RecA inhibitors effective for use as antibiotic agents or potentiating agents according to the present invention are those that can simultaneously bind to two distinct sites on RecA, for example to the ATP binding site and to another binding site, including for example one of the sites identified in Example 20.

Alternatively or additionally, it may be the case that, although many agents can inhibit the RecA ATPase, most such agents also inhibit other ATPases within a cell, causing a variety of stresses and inducing protective mechanisms (*e.g.*, shut down of DNA replication) that allow the cells to avoid the effects of antibiotic agents.

[00252] In some embodiments of the present invention, RecA inhibitors are small molecule agents, typically having some cyclic character (*e.g.*, including one or more aryl rings). Certain RecA inhibitors according to the present invention are flavones; certain RecA inhibitors according to the present invention are bisflavones. In some embodiments of the invention, the RecA inhibitor is or includes hinokiflavone.

[00253] In some embodiments of the present invention, RecA inhibitors are small molecule compounds that have the structure of formula I:



I

or a pharmaceutically acceptable salt or derivative thereof, wherein:

X is oxygen, sulfur, or N(R);

n is 0 to 4;

R^1 is hydrogen, or an optionally substituted group selected from a $C_{1\text{-}6}$ aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

each R^2 is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$;

each R^3 is independently hydrogen or an optionally substituted group selected from a $C_{1\text{-}6}$ aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched $C_{1\text{-}6}$ hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –

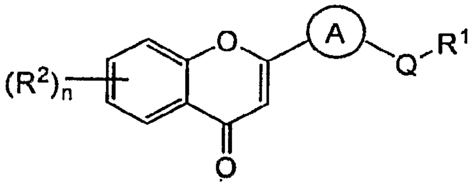
O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

each R is independently hydrogen or an optionally substituted aliphatic group;

R^x is R or OR; and

Ring A is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8–10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00254] As defined generally above, the X group of formula **I** is oxygen, sulfur, or N(R). In certain embodiments, the X group of formula **I** is oxygen. Accordingly, the present invention provides RecA inhibitors of formula **I-a**:



I-a

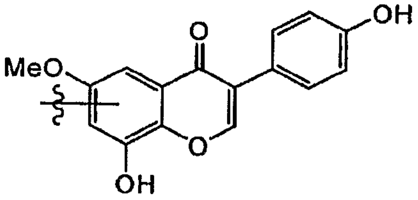
or a pharmaceutically acceptable salt or derivative thereof, wherein each of Ring A, Q, n, R^1, and R^2 are as defined above and described herein.

[00255] As defined generally above, the R^1 group of formula **I** is hydrogen, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00256] In certain embodiments, the R^1 group of formula **I** is a bicyclic 8–10 membered saturated, partially unsaturated, or aryl bicyclic ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In still other embodiments, R^1 is a bicyclic 10 membered partially unsaturated ring having one oxygen atom optionally substituted with 1 to 3 substituents independently selected from halogen, $-(CH_2)_{0-4}R^\circ$, $-(CH_2)_{0-4}OR^\circ$, $-(CH_2)_{0-4}SR^\circ$, $-(CH_2)_{0-4}Ph$, optionally substituted with R° or OR°, $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ optionally substituted with R° or OR°, -CH=CHPh, optionally substituted with R° or OR°,

-$(CH_2)_{0-4}N(R^\circ)_2$, wherein each R° may be substituted as defined herein and is independently hydrogen, C_{1-6} aliphatic, -CH_2Ph, -$O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to one embodiments, R^1 is substituted with 1 to 3 groups independently selected from OH and OMe.

[00257] According to one embodiment of the invention, the R^1 group of formula **I** is selected from:



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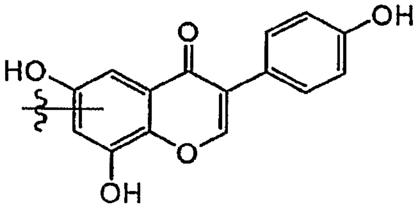

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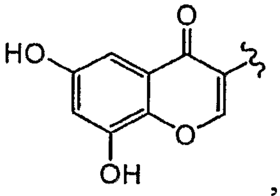

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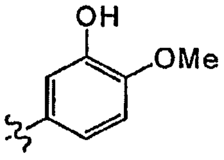
wherein each wavy line depicts the point of attachment to Q.

[00258] In certain embodiments, the R^1 group of formula **I** is a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, the R^1 group of formula **I** is a monocyclic 5-6 membered aryl ring having 0-2 nitrogen atoms, wherein R^1 is optionally substituted with 1 to 3 substituents independently selected from halogen, -$(CH_2)_{0-4}R^\circ$, -$(CH_2)_{0-4}OR^\circ$, -$(CH_2)_{0-4}SR^\circ$, -$(CH_2)_{0-4}Ph$, optionally substituted with R° or OR°, -$(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ optionally substituted with R° or OR°, -CH=CHPh, optionally substituted with R° or OR°, -$(CH_2)_{0-4}N(R^\circ)_2$, wherein each R° may be substituted as defined herein and is independently hydrogen, C_{1-6} aliphatic, -CH_2Ph, -$O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to one embodiment of the present invention, R^1 is phenyl substituted with 1 to 3 groups independently selected from halogen, -$(CH_2)_{0-4}R^\circ$, and -$(CH_2)_{0-4}OR^\circ$. Such groups include chloro, fluoro, OH, OMe, methyl, ethyl, propyl, cyclopropyl, isopropyl, and the like. In some embodiments, OH or OMe groups are present.

[00259] According to another embodiment, the R^1 group of formula **I** is selected from:



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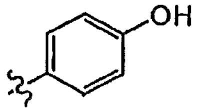

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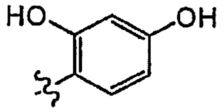

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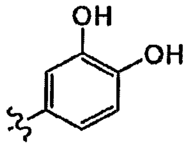

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wherein each wavy line indicates the point of attachment to Q.

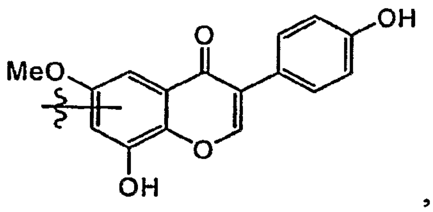
[00260] As defined generally above, the Q group of formula I is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In certain embodiments, Q is a valence bond such that R^1 is directly attached to Ring A. In other embodiments, Q is a bivalent, saturated, and straight C_{1-3} hydrocarbon chain, wherein 0-1 methylene units of Q is replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In still other embodiments, Q is -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. According to another embodiment, Q is -O-.

[00261] As defined generally above, the Ring A group of formula I is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, Ring A is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, Ring A is an optionally substituted 5-6 membered bivalent aryl ring having 0–2 nitrogen atoms. In still other embodiments, Ring A is phenylene optionally substituted with 1 to 4 groups independently selected from halogen, $-(CH_2)_{0-4}R^\circ$, $-(CH_2)_{0-4}OR^\circ$, $-(CH_2)_{0-4}SR^\circ$, $-(CH_2)_{0-4}Ph$, optionally substituted with R° or OR°, $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ optionally substituted with R° or OR°, -CH=CHPh, optionally substituted with R° or OR°, $-(CH_2)_{0-4}N(R^\circ)_2$, wherein each R° may be substituted as defined herein and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to another embodiment, Ring A is phenylene optionally substituted with 1-2 groups

independently selected from halogen, -$(CH_2)_{0-4}R^\circ$, and -$(CH_2)_{0-4}OR^\circ$. Such groups include chloro, fluoro, OH, OMe, methyl, ethyl, propyl, cyclopropyl, isopropyl, and the like. In some embodiments, OH and/or OMe group(s) are present.

[00262] As defined generally above, each R^2 group of formula **I** is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$, wherein each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8-10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, each R^2 group is independently halogen, R^3, OR^3, SR^3, or $N(R^3)_2$, wherein each R^3 is as defined above. According to one embodiment, at least one R^2 group is OH. According to another embodiment, one R^2 group is R^3 wherein R^3 is an optionally substituted bicyclic 8-10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In still other embodiments, one R^2 group is R^3 wherein R^3 is a bicyclic 10 membered partially unsaturated ring having one oxygen atom optionally substituted with 1 to 3 substituents independently selected from halogen, -$(CH_2)_{0-4}R^\circ$, -$(CH_2)_{0-4}OR^\circ$, -$(CH_2)_{0-4}SR^\circ$, -$(CH_2)_{0-4}Ph$, optionally substituted with R° or OR°, -$(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ optionally substituted with R° or OR°, -CH=CHPh, optionally substituted with R° or OR°, -$(CH_2)_{0-4}N(R^\circ)_2$, wherein each R° may be substituted as defined herein and is independently hydrogen, C_{1-6} aliphatic, -CH_2Ph, -$O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to one embodiments, R^3 is substituted with 1 to 3 groups independently selected from OH and OMe and optionally substituted phenyl.

[00263] According to some embodiments of the invention, the R^2 group formula **I** is OH, OMe, or is selected from:



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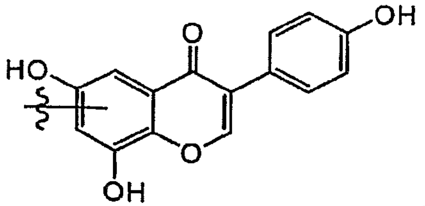

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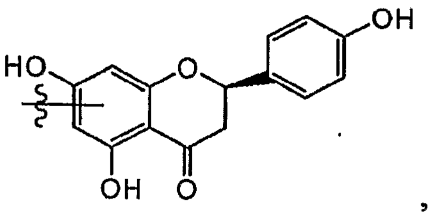

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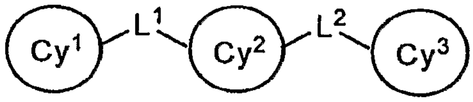
wherein each wavy line depicts the point of attachment to the phenyl group of formula I.

[00264] Exemplary compounds of formula **I** are set forth in Figures 15 and 16.

[00265] The present invention specifically identifies hinokiflavone as a particularly desirable RecA inhibitor within the scope of formula I. The present invention also encompasses the recognition that compounds that share structural (see, for example, Figure 15) and/or energetic (see, for example, Figure 16) features of hinokiflavone may be useful as RecA inhibitors as described herein. Thus, according to the present invention, compounds having the structures set forth in Figures 15 or 16 and/or having the structure of formula **II** may be useful as RecA inhibitors as described herein:



II

or a pharmaceutically acceptable salt or derivative thereof, wherein:

Cy^1 is a an optionally substituted 5-6 membered aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^1 is a valence bond, a C_{1-6} bivalent saturated, unsaturated, straight or branched hydrocarbon chain, $-N(R)-$, $-N(R)SO_2-$, $-N(R)SO_2N(R)-$, $-N(R)C(O)-$, $-C(O)N(R)-$, or $-N(R)C(O)N(R)-$;

each R is independently hydrogen or an optionally substituted C_{1-6} aliphatic group;

Cy^2 is an optionally substituted 6-membered aryl ring having 0-2 nitrogen atoms, an 8-10 membered bicyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 5-membered heteroaryl ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^2 is a C_{1-6} bivalent saturated, unsaturated, straight or branched hydrocarbon chain, $-CH_2CH_2C(=W)N(R)N(R)C(=W)-$, $-N(R)C(=W)N(R)C(=W)C(R)_2W-$, $-C(=W)N(R)N(R)C(=W)N(R)-$, $-C(=W)N(R)N(R)C(=W)N(R)CH=CH_2$, or $-C(=W)N(R)C(=W)N(R)-$, wherein each W is independently oxygen or sulfur; and

Cy^3 is an optionally substituted 6-membered aryl ring having 0-2 nitrogen atoms.

[00266] It should be understood that, unless otherwise stated, chemical structures or formulae depicted herein are also meant to include all isomeric (*e.g.*, enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, (Z) and (E) double bond isomers, and (Z) and (E) conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of depicted structures or formulae are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the structures or formulae of the invention are within the scope of the invention. Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the depicted structures or formulae except for the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention. Such compounds may be useful, for example, as analytical tools or probes in biological assays.

[00267] It should further be understood that the present invention encompasses pharmaceutically acceptable derivatives, and in particular prodrugs, metabolites, and pharmaceutically acceptable salts of the depicted compounds.

[00268] As discussed herein, inventive RecA inhibitors are desirably utilized in combination with one or more antibiotic agents, and particularly in combination with one or more antibiotic agents whose activity is potentiated by inventive RecA inhibitors and/or whose effectiveness can be reduced by development of resistance, which development is suppressed or retarded by inventive RecA inhibitors.

[00269] Exemplary structural classes of antibiotics for use in combination with RecA inhibitors according to the present invention include, but are not limited to, aminoglycosides, aminomethylcyclines, amphenicols, ansamycins, β-lactams (*e.g.*, penicillins or cephalosporins), carbapenems,. dapsones, 2,4-diaminopyrimidines, glycopeptides, glycycyclines, ketolides, lincomycins, lincosamides, macrolides, nitrofurans, oxazolidinones, peptides, polymyxins, quinolones, rifabutins, streptogramins, sulfonamides, sulfones, tetracyclines, and combinations thereof.

[00270] Exemplary mechanistic classes of antibiotics for use in combination with RecA inhibitors according to the present invention include, but are not limited to, those that inhibit protein synthesis, cell wall synthesis, DNA replication, transcription, and/or cell division. It

will be appreciated that biological and biochemical pathways are not mutually exclusive and that some biological or biochemical pathways may be considered to be subsets or sub-pathways of other biological or biochemical pathways. Mechanisms of action more specifically include, but are not limited to, inhibiting protein synthesis (*e.g.*, by binding ribosomal RNA or proteins, blocking tRNA binding to ribosome-mRNA complex, inhibiting peptidyl transferase), inhibiting or interfering with synthesis of a cell wall component (*e.g.*, inhibition of peptidoglycan synthesis, disruption of peptidoglycan cross-linkage, disruption of movement of peptidoglycan precursors, disruption of mycolic acid or arabinoglycan synthesis), cell membrane disruption, inhibiting or interfering with nucleic acid synthesis or processing, acting as "antimetabolites" and either inhibiting an essential bacterial enzyme or competing with a substrate of an essential bacterial enzyme, inhibiting or interfering with cell division.

[00271] It is understood by those of ordinary skill in the art that antibiotic agents of a particular structural class typically (though not necessarily) fall within the same mechanistic class.

[00272] As established herein and in related patent application serial number 60/772,648, the entire contents of which are incorporated herein by reference, RecA inhibitors can potentiate the activity of certain antibiotics. In particular, RecA inhibitors can potentiate the activity of quinolone antibiotics, *e.g.*, fluoroquinolones such as norfloxacin and ciprofloxacin. RecA inhibitors also suppress or retard resistance to quinolone antibiotics, *e.g.*, fluoroquinolines such as norfloxacin and ciprofloxacin.

[00273] Given the generality of the effect of RecA inhibition as it relates to the activity of and/or resistance to antibiotic agents, it is understood that RecA inhibitors that potentiate the activity of or suppress/retard resistance to a particular antibiotic agent are likely to similarly potentiate the activity of or suppress/retard resistance to other agents of the same structural and/or mechanistic class. Thus, demonstration of activity with regard to a particular fluoroquinolone, absent unusual circumstances, establishes the likelihood of activity with regard to other fluoroquinolones, and indeed with regard to quinolones generally. In fact, such demonstration can even establish the likelihood of activity with regard to other antibiotic agents generally, certainly for others with a common mechanism of action (*i.e.*, within the same mechanistic class).

[00274] Of course, it will be appreciated that an agent that potentiates the activity of or suppresses/retards resistance to any particular antibiotic agent within a particular structural or mechanistic class need not potentiate all, most, or any other members of that class, although in certain embodiments of the invention the agent does potentiate at least one, many, most, or all other members of that class.

[00275] Exemplary quinolone antibiotics include, but are not limited to, any of the antibacterial agents disclosed in the foregoing references including, but not limited to, ciprofloxacin, oxolinic acid, cinoxacin, flumequine, miloxacin, rosoxacin, pipemidic acid, norfloxacin, enoxacin, moxifloxacin, gatifloxacin, ofloxacin, lomefloxacin, temafloxacin, fleroxacin, pefloxacin, amifloxacin, sparfloxacin, levofloxacin, clinafloxacin, nalidixic acid, enoxacin, grepafloxacin, levofloxacin, lomefloxacin norfloxacin, ofloxacin, trovafloxacin, olamufloxacin, cadrofloxacin, alatrofloxacin, gatifloxacin, rufloxacin, irloxacin, prulifloxacin, pazufloxacin, gemifloxacin, sitafloxacin, tosulfloxacin, amifloxacin, nitrosoxacin-A, DX-619, and ABT-492. Quinolone antibiotics include fluoroquinolones (*e.g.*, having a fluorine substituent at the C-6 position), and non-fluoroquinolones. Also included within the scope of quinolone antibiotics are derivatives in which a quinolone is conjugated with, e.g., covalently bound to, another core structure. For example, U.S. Pub. No. 2004-0215017 discloses compounds in which an oxazolidinone, isoxazolinone, or isoxazoline is covalently bonded to a quinolone.

[00276] Included within the scope of quinolone antibiotics that can be utilized in accordance with the present invention are compounds that have a core structure related to the 4-oxo-1,4-dihydroquinoline and 4-oxo-1,4 dihydronapthyridine systems, *e.g.*, 2-pyridones, 2-naphthyridinones, and benzo[b]napthyridones. 2-pyridones are potent inhibitors of bacterial type II topoisomerases (Saiki *et al.*, Antimicrob. Agents Chemother., 1999, 43: 1574). The core structures are depicted in Figure 9.

[00277] Also included within the scope of quinolone antibiotics that can be potentiated are compounds that have core structures related to the quinolone core structures depicted in Figures 9 or 10. Certain of these core structures are shown in Figures 11A or 11B and references thereto are provided in Ronald and Low (Eds.), "*Fluoroquinolone Antibiotics*", Birkhäuser Verlag, Basel, 2003; DaSilva *et al.*, Curr. Med. Chem., 2003, 10: 21.

[00278] The invention encompasses the use of RecA inhibitors as described herein to potentiate the activity of and/or to suppress/retard the resistance to, these antibiotic agents.

[00279] As noted above, RecA inhibitors that potentiate the activity of or suppress /retard to an antibiotic agent within a particular mechanistic class are likely to also potentiate the activity of or suppress/retard resistance to other antibiotic agents within the same mechanistic class. Thus, RecA inhibitors that potentiate the activity of and/or suppress/retard resistance to particular quinolones are likely to also potentiate the activity of and/or suppress/retard resistance to certain other topoisomerase II inhibitors, some of which are structurally related to quinolones. Exemplary topoisomerase II inhibitors that bind to GyrB include the coumarins, novobiocin and coumermycin A1, cyclothialidine, cinodine, and clerocidin. Additional agents that are reported to bind to and/or inhibit gyrase, topoisomerase IV, or both, are disclosed in U.S. Pat. Nos. 6,608,087 and 6,632,809 and in U.S. Pub. Nos. 20040043989 and 20050054697. The present invention encompasses the use of RecA inhibitors to potentiate the activity of and/or to suppress/retard resistance to any of these agents. That is, the present invention provides RecA inhibitors for use in combination with any of these compounds or any compound or agent that inhibits one or more microbial type II topoisomerases.

VI. Potentiating Cidal Antibiotics *via* RecA Inhibition

[00280] Current antimicrobial therapies, which cover a wide array of targets (C. Walsh, Nature Rev. Microbiol., 2003, 1: 65-70), fall into two general categories: bactericidal drugs which kill bacteria with an efficiency of >99.9% and bacteriostatic drugs which merely inhibit growth (G.A. Pankey and L.D. Sabath, Clin. Infect. Dis., 2004, 38: 864-870). Antibacterial drug-target interactions are well-studied and predominantly fall into three classes: inhibition of DNA replication and repair, inhibition of protein synthesis, and inhibition of cell-wall turnover (C. Walsh, Nature, 2000, 406: 75-781). However, our understanding of many of the bacterial responses that occur as a consequence of the primary drug-target interaction remains incomplete (A. Tomasz, Annu. Rev. Microbiol., 1979, 33: 113-137; K. Drlica and X. Zhao, Microbiol. Mol. Biol. Rev., 1997, 61: 377-392; B.D. Davis, Microbiol. Rev., 1987, 51: 341-350; K. Lewis, Microbiol. Mol. Biol. Rev., 2000, 64: 503-514).

[00281] The present Applications have shown (see Example 39) that the three major classes of bactericidal antibiotics, regardless of drug-target interaction, stimulate the production of highly deleterious hydroxyl radicals in gram-negative and gram-positive bacteria, which ultimately contribute to cell death. The Applicants have shown that, in

contrast, bacteriostatic drugs do no produce hydroxyl radicals, and that the mechanism of hydroxyl radical formation, induced by bactericidal antibiotics, involves the tricarboxylic acid cycle and a transient depletion of NADH. The results obtained suggest that all three major classes of bactericidal drugs can be potentiated by targeting bacterial systems that remediate hydroxyl radical damage, including proteins involved in triggering the DNA damage response, *e.g.*, RecA.

[00282] Accordingly, the present invention provides methods that involve the use of RecA inhibitors to potentiate cidal antibiotics, and/or any therapeutic agent that produces hydroxyl radicals. Such methods are suitable for use with any member of a large variety of classes of antibiotics mentioned herein, as long as such member produces hydroxyl radicals.

VII. Applications

[00283] As noted above, the target genes of the invention may be found in many organisms, including bacteria of any one or more types, *e.g.*, Gram negative bacteria, Gram positive bacteria, and/or acid fast bacteria. Antibiotic potentiating agents and compositions containing them may accordingly be used to inhibit growth of bacteria of a wide variety of types including, but not limited to, members of any bacterial genus or species mentioned above.

[00284] The antibiotic potentiating agents and compositions containing them can be used to inhibit bacterial growth and/or survival in a variety of contexts. For example, they may be employed to inhibit growth and/or survival of bacteria maintained in cell culture or inhabiting locations in the environment, *e.g.*, inert surfaces, clothing, towels, bedding, utensils, etc. Of particular interest are fomites, *i.e.*, inanimate objects that may be contaminated with disease-causing microorganisms and may serve to transmit disease to a human or animal. Such locations or objects can be contacted with a solution containing the potentiating agent and an antibiotic that it potentiates. The antibiotic potentiating agents, antibiotics that they potentiate, and/or compositions containing them can be added to food or water, particularly for the prevention of bacterial disease in animals.

[00285] An antibiotic agent and an agent that potentiates the antibiotic (*e.g.*, a quinolone antibiotic and a quinolone potentiating agent), may be administered in combination to a subject in need thereof, *e.g.*, a human or animal suffering from or at risk of a bacterial infection. The antibiotic agent and the antibiotic potentiating agent, may be components of a single pharmaceutical composition or may be administered as individual pharmaceutical compositions. They may be administered using the same route of administration or different routes of administration. In certain embodiments of the invention a unit dosage form containing a predetermined amount of an antibiotic and a predetermined amount of an antibiotic potentiating agent is administered.

[00286] A therapeutic regimen that includes an antibiotic and an antibiotic potentiating agent may (i) allow the use of a reduced daily dose of the antibiotic without significantly reducing efficacy; (ii) allow the use of a shorter course of administration of the antibiotic without significantly reducing efficacy; (iii) be effective against a microorganism species or strain that would otherwise be resistant to the antibiotic when used at clinically tolerated doses, *e.g.*, conventional doses.

[00287] Infections and infection-related conditions that can be treated using an antibiotic potentiating agent and an antibiotic include, but are not limited to, pneumonia, meningitis, sepsis, septic shock, sinusitis, otitis media, mastoiditis, conjunctivitis, keratitis, external otitis (*e.g.*, necrotizing otitis externa and perichondritis), laryngeal infections (*e.g.*, acute epiglottitis, croup and tuberculous laryngitis), endocarditis, infections of prosthetic valves, abscesses, peritonitis, infectious diarrheal diseases, bacterial food poisoning, sexually transmitted diseases and related conditions, urinary tract infections, pyelonephritis, infectious arthritis, osteomyelitis, infections of prosthetic joints, skin and soft tissue infections, oral infections, dental infections, nocardiosis and actinomycosis, mastitis, brucellosis, Q fever, anthrax, wound infections, etc.

[00288] In certain embodiments of the invention an antibiotic potentiating agent and an antibiotic that it potentiates are used to treat or prevent infection associated with an indwelling device. Indwelling devices include surgical implants, prosthetic devices, and catheters, *i.e.*, devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, defibrillators, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, continuous ambulatory peritoneal dialysis (CAPD) catheters, spinal rods, implantable pumps for medication delivery, etc. Potentiating agents identified by the methods of the invention can be applied to, coated on, imbedded in, or otherwise combined with an indwelling device to prophylactically prevent infections, optionally together with an antibiotic. Alternatively, an agent of the invention may be administered to a subject, *e.g.*, by injection to achieve a systemic effect shortly before insertion of an indwelling device. The antibiotic to be potentiated could be applied to, coated on, imbedded in, or otherwise combined with an indwelling device or may also be delivered systemically. Of course local delivery of the potentiating agent and/or antibiotic may also be employed. Treatment may be continued after implantation of the device during all or part of the time during which the device remains in the body and, optionally, thereafter. Agents of this invention may be used in combination with an antibiotic prophylactically prior to dental treatment or surgery.

[00289] Alternatively, an agent of this invention and an antibiotic that it potentiates can be used to bathe an indwelling device immediately before insertion and/or to bathe wounds or sites of insertion. Exemplary concentrations useful for these purposes range between 1 μg/ml to 10 μg/ml for bathing of wounds or indwelling devices.

[00290] Diagnostic methods for determining whether a subject is suffering from or at risk of a microbial infection are well known in the art, and any such method can be used to identify a suitable subject for administration of an antibiotic and an agent that potentiates the antibiotic. Methods include clinical diagnosis based at least in part on symptoms, imaging studies, immunodiagnostic assays, nucleic acid based diagnostics, and/or isolation and culture of potentially causative microorganisms from samples such as blood, urine, sputum, synovial fluid, cerebrospinal fluid, pus, or any sample of body fluid or tissue. The inventive methods can include a step of identifying a subject suffering from or at risk of a microbial infection, a step of identifying a microorganism suspected of causing the infection, a step of selecting a therapeutic regimen based at least in part on the identity or suspected identity of the microorganism and/or the location or characteristics of the infection. In certain embodiments of the invention the method includes determining that the subject has a significant likelihood (*e.g.*, at least 5%) of suffering from or being at risk of infection by a microorganism that is resistant to one or more antibiotics and that antibiotic potentiation is advisable.

[00291] A subject is "at risk of" an infection in any of a variety of circumstances. "At risk of" implies at increased risk of, relative to the risk such subject would have in the absence of one or more circumstances, conditions, or attributes of that subject, and/or (in the case of humans) relative to the risk that an average, healthy member of the population would have. Specific examples of conditions that place a subject "at risk" include, but are not limited to, immunodeficiencies (particularly those affecting the humoral or non-specific (innate) immune system), prior treatment with antibiotics that may have reduced or eliminated normal microbial flora, treatment with agents that suppress the immune system (*e.g.*, cancer chemotherapy, immunosuppressive agents), chronic diseases such as diabetes or cystic

fibrosis, surgery or other trauma, infancy or old age, occupations or living conditions that entail exposure to pathogenic microorganisms, etc.

[00292] While it is anticipated that the antibiotic potentiating agent identified according to the inventive methods will find particular use for inhibiting the growth and/or survival of microorganisms, they may also be used for other purposes. For example, certain of the target genes identified herein may have homologs in multicellular organisms, *e.g.*, animals or humans, and such homologs may themselves be targets for treatment of disease in those organisms. Alternatively or additionally, an agent identified according to a method of the invention may potentiate a therapeutic agent used in treating a disease other than a microbial infection. Agents that inhibit mammalian topoisomerases are of use for the treatment of a variety of cancers. Exemplary agents include camptothecins (*e.g.*, irinotecan and topotecan) and edotecarin (which inhibit mammalian type I topoisomerase), and etoposide (a mammalian type II topoisomerase inhibitor). Without wishing to be bound by any theory, agents that potentiate a microbial topoisomerase inhibitor may also potentiate an agent that inhibits mammalian topoisomerase. Such agents may therefore be of use in cancer chemotherapy regimens that employ a mammalian topoisomerase inhibitor.

VIII. Pharmaceutical Compositions and Kits

[00293] Suitable preparations, *e.g.*, substantially pure preparations of the agents described herein may be combined with pharmaceutically acceptable carriers, diluents, solvents, excipients, etc., to produce an appropriate pharmaceutical composition. The invention therefore provides a variety of pharmaceutically acceptable compositions for administration to a subject comprising (i) an antibiotic potentiating agent; and (ii) a pharmaceutically acceptable carrier or excipient. The invention further provides a pharmaceutically acceptable composition comprising (i) an antibiotic potentiating agent; (ii) an antibiotic whose activity is potentiated by the compound; and (iii) a pharmaceutically acceptable carrier or excipient. The invention further provides a pharmaceutically acceptable unit dosage form containing a predetermined amount of an antibiotic and a predetermined amount of an antibiotic potentiating agent, wherein the predetermined amounts are selected so that the antibiotic potentiating agent potentiates the antibiotic when the unit dosage form is administered to a subject. In certain embodiments, the antibiotic is a quinolone antibiotic. In other embodiments, the antibiotic is an aminoglycoside. In still other embodiments, the antibiotic is a lactam (*e.g.*, a β-lactam). In certain embodiments, the antibiotic potentiating agent is a RecA inhibitor.

[00294] In certain embodiments of the invention the pharmaceutical composition is a sustained release formulation. A variety of methods are known in the art for achieving sustained release, *e.g.*, by prolonging residence time in the stomach (such as through the use of swellable polymers), providing pH or enzyme-sensitive coatings, employing bioadhesive coatings that stick to the walls of the stomach or intestine, etc. See, *e.g.*, U.S. Pub. No. 2004-0024018 and references therein.

[00295] It is to be understood that the pharmaceutical compositions of the invention, when administered to a subject, are preferably administered for a time and in an amount sufficient to treat or prevent the disease or condition for whose treatment or prevention they are administered, *e.g.*, a bacterial infection.

[00296] Further provided are pharmaceutically acceptable compositions comprising a pharmaceutically acceptable derivative (*e.g.*, a prodrug) of any of the agents of the invention, by which is meant any non-toxic salt, ester, salt of an ester or other derivative of a agent of this invention that, upon administration to a recipient, is capable of providing, either directly or indirectly, an agent of this invention or an inhibitorily active metabolite or residue thereof. As used herein, the term "inhibitorily active metabolite or residue thereof" means that a metabolite or residue thereof exhibits inhibitory activity towards a protein or microorganism. The inhibitor of a bacterial type II topoisomerase may also be provided as a prodrug.

[00297] In various embodiments of the invention an effective amount of the pharmaceutical composition is administered to a subject by any suitable route of administration including, but not limited to, intravenous, intramuscular, by inhalation (*e.g.*, as an aerosol), by catheter, intraocularly, orally, rectally, intradermally, by application to the skin, etc.

[00298] The term "pharmaceutically acceptable carrier, excipient, or vehicle" refers to a non-toxic carrier, excipient, or vehicle that does not destroy the pharmacological activity of the agent with which it is formulated. Pharmaceutically acceptable carriers, excipients, or vehicles that may be used in the compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl

pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration may be included. Supplementary active compounds, *e.g.*, compounds independently active against the disease or clinical condition to be treated, or compounds that enhance activity of a compound, can also be incorporated into the compositions.

[00299] Pharmaceutically acceptable salts of the agents of this invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

[00300] Salts derived from appropriate bases include alkali metal (*e.g.*, sodium and potassium), alkaline earth metal (*e.g.*, magnesium), ammonium and NNo(C1-4 alkyl)4 salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization.

[00301] A pharmaceutical composition is formulated to be compatible with its intended route of administration. Solutions or suspensions used for parenteral (*e.g.*, intravenous), intramuscular, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as

acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00302] Pharmaceutical compositions suitable for injectable use typically include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ), phosphate buffered saline (PBS), or Ringer's solution.

[00303] Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. . Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[00304] In all cases, the composition should be sterile, if possible, and should be fluid to the extent that easy syringability exists.

[00305] Preferred pharmaceutical formulations are stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. In general, the relevant carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various

antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin. Prolonged absorption of oral compositions can be achieved by various means including encapsulation.

[00306] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Preferably solutions for injection are free of endotoxin. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00307] Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. Formulations for oral delivery may advantageously incorporate agents to improve stability within the gastrointestinal tract and/or to enhance absorption.

[00308] For administration by inhalation, the inventive compositions are preferably delivered in the form of an aerosol spray from a pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer. Liquid or

dry aerosol (*e.g.*, dry powders, large porous particles, etc.) can be used. The present invention also contemplates delivery of compositions using a nasal spray.

[00309] For topical applications, the pharmaceutically acceptable compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutically acceptable compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2–octyldodecanol, benzyl alcohol and water.

[00310] For local delivery to the eye, the pharmaceutically acceptable compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutically acceptable compositions may be formulated in an ointment such as petrolatum.

[00311] The pharmaceutically acceptable compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[00312] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00313] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00314] In addition to the agents described above, in certain embodiments of the invention, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polyethers, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Certain of the materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811 and other references listed herein. Liposomes, including targeted liposomes (e.g., antibody targeted liposomes) and pegylated liposomes have been described (Hansen CB, et al., *Biochim Biophys Acta.* 1239(2):133-44,1995; Torchilin VP, et al., *Biochim Biophys Acta,* 1511(2):397-411, 2001; Ishida T, et al., *FEBS Lett.* 460(1):129-33, 1999). One of ordinary skill in the art will appreciate that the materials and methods selected for preparation of a controlled release formulation, implant, etc., should be such as to retain activity of the compound. For example, it may be desirable to avoid excessive heating of polypeptides, which could lead to denaturation and loss of activity.

[00315] It is typically advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00316] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit high therapeutic indices are preferred. While compounds that

exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00317] The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose (*e.g.*, dose that is therapeutically effective to achieve a desired degree of antibiotic potentiation) can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*e.g.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms, half-maximal inhibition of growth or survival of an infectious agent, etc.) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

[00318] A therapeutically effective amount of a pharmaceutical composition typically ranges from about 0.001 to 100 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The pharmaceutical composition can be administered at various intervals and over different periods of time as required, *e.g.*, multiple times per day, daily, every other day, once a week for between about 1 to 10 weeks, between 2 to 8 weeks, between about 3 to 7 weeks, about 4, 5, or 6 weeks, etc. The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Generally, treatment of a subject with an inventive composition can include a single treatment or, in many cases, can include a series of treatments. It will be appreciated that a range of different dosage combinations (*i.e.*, doses of the antibiotic and antibiotic potentiating agent) can be used.

[00319] Exemplary doses include milligram or microgram amounts of the inventive compounds per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram.) For local administration (*e.g.*, intranasal), doses much smaller than these may be used. It is furthermore understood that appropriate doses depend upon the potency of the agent, and may optionally be tailored to the particular recipient, for example, through administration of increasing doses until a preselected desired response is achieved. It is understood that the specific dose level for any particular subject may depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

[00320] The invention further provides pharmaceutical compositions comprising two or more compounds of the invention and, optionally, one or more antibiotic agents. The invention further provides pharmaceutical compositions comprising one or more compounds of the invention, optionally one or more antibiotic agents, and an additional active agent. The additional active agent may be an antibiotic that has a different mechanism of action to that of the antibiotic that is potentiated by the compound.

[00321] The present invention also provides pharmaceutical packs or kits comprising one or more containers (*e.g.*, vials, ampoules, test tubes, flasks, or bottles) containing one or more ingredients of the inventive pharmaceutical compositions, for example, allowing for the simultaneous or sequential administration of the antibiotic potentiating agent and antibiotic agent(s) it potentiates. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Different ingredients may be supplied in solid (*e.g.*, lyophilized) or liquid form. Each ingredient will generally be suitable as aliquoted in its respective container or provided in a concentrated form. Kits may also include media for the reconstitution of lyophilized ingredients. The individual containers of the kit are preferably maintained in close confinement for commercial sale.

Equivalents and Scope

[00322] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the

above Description and Examples below, but rather is as set forth in the appended claims. In the claims and elsewhere in the specification, articles such as "a,", "an" and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims (or from the portion of the specification relevant to such claim or claim element) is introduced into another claim. For example, and without limitation, any claim that is dependent on another claim can be modified to include one or more elements or limitations found in any other claim (or from the portion of the specification relevant to such claim or claim element) that is dependent on the same base claim. Furthermore, where the claims or description recite a composition, it is to be understood that methods of administering the composition according to any of the methods disclosed herein, and methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[00323] Where elements are presented as lists, *e.g.*, in Markush group format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should it be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not been specifically set forth *in haec verba* herein.

[00324] The inclusion of a "providing a subject..." step in certain methods of the invention is intended to indicate that the composition is administered to treat a bacterial

infection. Thus the subject will have or be at risk of a bacterial infection and the composition is administered to treat the infection, typically upon the sound recommendation of a health care provider, who may or may not be the same individual who administers the composition. The invention includes embodiments in which a step of providing is not explicitly included and embodiments in which a step of providing is included.

[00325] Where ranges are given in the instant specification, including the claims, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00326] Any particular embodiment of the invention (*e.g*,. any antibacterial agent, any bacterial target, any compound, any method of treatment etc.) can be excluded from any one or more claims, for any reason. In particular, and without limiting the preceding sentence, any embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein.

Examples

Example 1: Identification of Mutations that Potentiate Quinolone Activity Using a Growth Assay

[00327] In order to discover genetic targets for quinolone potentiating agents the present Applications have established a screen to identify *E. coli* mutants that would be largely unable to grow in the presence of a concentration of quinolone that would not allow the growth of wild type *E. coli*. They decided to initially focus on two important members of this compound class, *i.e.*, norfloxacin (Nor) and ciprofloxacin (Cipro). The growth of wild type *E. coli* was first shown to be fully inhibited by 0.1 μg/mL of Nor (a lethal concentration) but not by 0.05 μg/mL (a sublethal concentration). While the latter dose was shown to activate the SOS response using microarray analysis (data not shown), it allowed bacterial growth to 80% of wild type levels. The wild type *E. coli* was also shown to be able to grow in the presence of 0.1 μg/mL of Cipro (a sublethal concentration).

[00328] 0.05 μg/mL of Nor and Cipro at 0.010 μg/mL were used as sublethal concentrations to screen a set of approximately 4,200 *E. coli* mutants, each having a deletion (knock-out) of a single gene. Each deletion strain was constructed by replacing the chromosomal copy of the relevant gene in a wild type *E. coli* strain (K-12) with a selectable antibiotic resistance gene, essentially as described, to create a precise gene deletion (Datsenko, K.A. and Wanner, B.L., "One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products", *Proc Natl Acad Sci U S A*, 97, 6640-6645, 2002); Baba, T., Ara, T., Okumura, Y., iki Hasegawa, M., Takai, Y., Baba, M., Oshima, T., Tomita, M., Wanner, B. and Mori, H. (2003) Systematic construction of single gene deletion mutants in Escherichia coli K-12 (in preparation) and see also the web site at ecoli.aist-nara.ac.jp/gb5/Resources/deletion/deletion.html). The entire deletion set was screened 3 times with Nor and 2 times with Cipro, with the goal of identifying mutants unable to grow at sublethal concentrations of either or both of these drugs.

[00329] For each mutant, the ratio of growth of the mutant in the presence of a sublethal concentration of quinolone to growth of the mutant in the absence of quinolone was calculated after 12 hours of growth at 37°C, based on OD_{600} measurements. From these studies, 36 strains were identified as being completely unable to grow in the presence of sublethal concentrations of Nor and/or Cipro relative to their ability to grow in the absence of the respective quinolone. Out of this screen *tolC* was identified as one of the genes whose deletion resulted in an inability to grow in the presence of a sublethal concentration of quinolone. *tolC* encodes for an efflux pump and its loss of function was shown to increase sensitivity to drugs (*e.g.*, Husain et al., 2004; reviewed in Koronakis, 2003). Therefore, this result was expected and was a validation of the ability of the screen to successfully identify genes whose mutation results in increased sensitivity to quinolones. These genes are therefore targets for the development of quinolone potentiating agents. Further details of the screen are provided below:

Protocol

[00330] **K12 *E. coli* whole genome deletion stocks.** The whole genome deletion set was obtained as a frozen stock in 90, 96 well plates containing LB, Kan 50 μg/mL. and 10% glycerol. The frozen stock was inoculated into new plates containing 150 μL of LB Kan 50 μg/mL per well using a 96 – pin replicator ((VP scientific, VP407A). These plates were grown statically overnight at 37°C.

[00331] **Preparing the assay plates.** 90, 96-well flat bottom polystyrene plates were filled with 150 μL of LB per well. 90 96-well flat polystyrene plates were filled with 150 μL of LB, Norfloxacin 25 μg/mL per well. 180 96-well flat polystyrene plates were filled with 150 μL of sterile, deionized water.

[00332] **Inoculating the assay plates.** The optimal inoculation is a 1000:1 dilution of an overnight growth culture. Each pin holds ~15 μL of culture so the pin tool must be used to serially dilute 3 times. The replicator was used to transfer the overnight mutant growth plates to the sterile water plate (dilution plate 1). The replicator was sterilized by washing in 70% ethanol, then 95% ethanol, then flamed. The replicator was again used to transfer from dilution plate 1 to a second (dilution plate 2). After sterilizing, the replicator was again used to transfer from dilution plate 2 to an LB plate. The replicator was again used to transfer from dilution plate 2 to an LB, Norfloxacin 25 ng/mL plate). The OD600 was measured for a blank plate to determine OD600 for the plate and media alone. The inoculated plates were grown statically overnight at 37°C.

[00333] **Result analysis.** After overnight growth, the OD600 was measured for every plate. The BLANKED OD600 was obtained for every culture by subtracting the average OD600 value from the blank plate. The RATIO of OD600 in the presence of compound relative to the no drug control for that plate was calculated for every culture. (compound OD 600 / no drug OD600).

OVERVIEW 384 well plate

[00334] 12- 384 well plates were filled with 50 μL of LB using the Beckman Multimek robot. The above plates were inoculated from the partially thawed glycerol stock plates using the VP384 replicator. The replicator is dipped in 70% ethanol for 10 seconds, 100% ethanol and then flamed in between plates. The cultures are grown statically overnight in the plates at 37°C with a plastic wrapping to prevent desiccation. 24 dilution plates are filled with 50 μL of sterile dH_2O for dilution of the cultures before stamping. Before the drug is added, a sample of the culture is taken and diluted into the above water plates using the VP384 replicator. The replicator holds 0.2 μL of liquid. This corresponds to a 1:250 dilution. Using the Multimek, 5 μL of LB Norfloxacin (10 μg/mL) was added to every well of every growth plate. While the drug treatment was incubating, the 0 drug time point dilution can be stamped on 245mm bioassay dishes using the VP384. (The plates must be sufficiently dry for this to work properly.) Two plates stamped in duplicate will fit on one

245mm assay dish. After a 3 hour incubation with Norfloxacin, another sample can be diluted in H_2O as described above. The 3 hour time point can then be stamped on the agar plates as described above. No washing is necessary because the cells are diluted down to 4 ng/mL of Norfloxacin. This is less than 1/ 25th MIC.

Example 2: Identification of Mutations that Potentiate Quinolone Activity Using a Survival Assay

[00335] 188 *E. coli* genes were identified for which a homolog existed in *S. aureus* (MRSA 252) by performing searches of publicly available sequence databases. Homologs were identified using the NCBI Genplot pairwise genome comparison of protein homologues. A 96 well plate format was then used to perform a survival assay on a set of *E. coli* deletion strains, each of which had a deletion in one of these highly conserved genes. The survival assay measured the number of cells which form colonies (colony forming units, or CFU) after growth in the presence of a compound. This assay was applied to identify deletion strains which cannot survive following a limited time period of exposure to a quinolone treatment at a lethal concentration as compared to survival of the parent strain. The lethal concentration one that inhibits growth and would eventually be lethal if exposure was continued indefinitely. 2 μg/mL of Nor was selected as the concentration to use.

[00336] The assay was performed as follows: Cultures were grown to early log phase in LB medium at 37°C with shaking. Nor (2 μg/mL) was added to the cultures, and incubation was continued for 60 minutes. Samples of the cells were collected and washed in PBS at 0 min (untreated) and 60 minutes after adding Nor. The reduction in number of viable cells that occurred as a result of exposure to Nor was determined by stamping identical 10-fold serial dilutions of the treated cells from each time point on LB agar as shown in Figure 2. Columns 1 and 12 represent wild type bacteria.

[00337] A number of strains were identified that displayed significantly reduced survival following exposure to Nor relative to survival of the parental (wild type) strain. For example, Fig. 2 shows that the *recA* null mutant, which was unable to grow in the presence of a sublethal concentration of Nor, was also unable to survive the Nor treatment. Thus in the left panel of Fig. 2, the *recA* deletion mutant (column 5) shows growth comparable to that of the wild type strain (columns 1 and 12) whereas cells of this strain show very poor growth following treatment with Nor (compare uppermost spot in column 5, left panel (untreated) with uppermost spot in column 5, right panel). However, the *tolC* mutant (column 9 in Fig. 2) was shown not to have a significant decrease in survival and behaved more similarly to the wild type strain.. A subset of the strains showing poor survival, including the *recA* deletion strain, had also been identified using the growth assay described in Example 1, thus providing further evidence of the significance of the deleted genes as targets for the identification of compounds that potentiate the activity of quinolone.

[00338] The *recA* null mutant was tested using the survival assay with Cipro rather than Nor and the mutant was observed to display reduced survival following exposure to a lethal concentration of Cipro (500 ng/mL).

Example 3: recA Mutant Response to Nor and Cipro

[00339] As described in Examples 1 and 2, deletion of *recA* in *E. coli* was shown to result in an inability to grow in the presence of sublethal concentrations of either Nor or Cipro (Example 1) and a reduced survival following exposure to lethal concentrations of Nor or Cipro (Example 2). The present Applicants decided to focus on *recA* as an exemplary target gene. Loss of recA function was first confirmed not to alter growth of *E. coli* (data not shown). The ability of Nor-sensitive and Nor-resistant wild type *E. coli* and *recA* deletion mutants to survive in the presence of Nor was then tested using the survival assay described in Example 2.

[00340] Nor-resistant *E. coli* strains were then constructed by introducing mutations into the *gyrA* locus using a standard molecular genetics approach (Datsenko and Wanner, 2000) and selecting for cells able to form colonies on plates containing Nor. The transformants were plated on LB plus Nor at 0.1 μg/mL. The individual colonies were streaked for single colonies on LB plus Nor at 0.1 μg/ml. The final selected candidates could form colonies at 0.175 μg/mL of Nor. Mutations in GyrA at position 83 and 87 are known to confer resistance to quinolones (Everett et al., 1996; Komp Lindgren et al., 2003). A PCR product containing a portion of *gyrA* and harboring mutations that result in a serine to leucine change at position 83 and an aspartic acid to asparagine change at position 87 in the GyrA polypeptide was transformed into MG1655 *recA*$^+$ (wild type strain) and MG1655 *recA*$^-$ (*recA* deletion strain) cells, which were then plated on medium containing Nor for selection. Transformants able to survive on this medium were selected and further characterized.

[00341] The ability of these fours strains to survive exposure to Nor was then tested. The experiment was performed as follows. Cultures were grown to early log phase in LB at 37°C with shaking. At the 0 time point Nor was added to the cultures to a final concentration of 0.1 μg/mL. This concentration of Nor is just below the MIC for strain MG1655 under these growth conditions. Samples of the cells were collected and washed in PBS at 0, 30, 90 and 180 min time points. The number of viable cells was determined by plating the cells from each time point on LB agar and counting colonies. The CFU counts were normalized based on the time 0 time point inoculum for each culture (*i.e.*, the CFU count obtained from the 0 time point inoculum was taken to represent 100% survival) and the log %survival was plotted (Figure 3).

[00342] Both of the *E. coli* *recA*$^-$ strains were observed to be highly sensitive to quinolone treatment and showed very poor viability following exposure to a sublethal concentration of the drug. As shown in Figure 3, far fewer *recA*$^-$ cells than *recA*$^+$ cells were able to form colonies after treatment with Nor. The *recA* deletion caused a 10,000 fold increase in quinolone sensitivity in a wild type background after 3 hours of exposure to Nor. In addition, deletion of *recA* in the strain background containing the mutation that confers Nor resistance on wild type *E. coli* caused the strain to exhibit wild type levels of sensitivity to Nor, *i.e.*, it rendered a strain that would otherwise have been Nor-resistant sensitive to Nor. *recA* was concluded to be essential for survival in the presence of quinolones in both wild-type and Nor resistant strain backgrounds. Based on these results, inhibiting the expression and/or activity of *E. coli* RecA (or its homolog in other bacteria) is expected to increase the sensitivity of the bacteria to quinolones to which they already display sensitivity (*i.e.*, will reduce the MIC), thereby allowing the use of a lower dose of the drug for treating subjects suffering from or at risk of bacterial infection. In addition, inhibiting the expression and/or activity of *E. coli* RecA (or its homolog in other bacteria) is expected to render bacterial strains that have acquired resistance to quinolones (*e.g.*, due to mutation in one or more subunits of the bacterial gyrase or topoisomerase IV and/or due to increased copy number of a gene encoding one of these polypeptides) sensitive once again to these compounds.

Example 4: RecA Deletion Increases Sensitivity of Staphylococcus aureus to Quinolones in Both Wild Type and Quinolone Resistant Strain Backgrounds

Growth assay

[00343] The growth and response to quinolone of *S. aureus* strain RN4220 (wild type) and its *recA*$^-$ derivative (RN4220 *recA*$^-$) were first examined. These strains have been described in the literature (Bayles *et al.*, 1994). The RN4220 *recA* null mutant (RN4220

recA$^-$) contains the *recA569* allele and is referred to KB103 in Bayles, *et al.*, 1994. RN4220 *recA*$^-$ was shown to be affected in genetic recombination and DNA repair (Bayles *et al.*, 1994). The present Applicants first showed that RN4220 *recA*$^-$ and the parental strain RN4220 have similar growth curves as measured by optical density (OD_{600}) or colony formation when grown in rich liquid medium in the absence of quinolone (not shown). They then determined that the MICs for Nor for RN4220 and RN4220 *recA*$^-$ were 1.53 and 0.38 µg/mL, respectively, indicating that loss of RecA function increases sensitivity to Nor. MICs were determined as described above.

[00344] Figure 4 summarizes the effect of deletion of either *recA* or *tolC* on bacterial growth in the presence of Nor (left panel) and survival following a period of exposure to Nor (right panel). Both *recA*$^-$ and *tolC*$^-$ strains showed a reduced MIC. However, only the *recA*$^-$ strain showed a decrease in survival while the *tolC*$^-$ strain has a survival response similar to that of the parent strain. Without wishing to be bound by any theory, this result suggests that inhibitors of RecA or other target polypeptides whose absence or inactivation inhibits both growth and survival may have advantages relative to inhibitors of bacterial efflux pumps.

Survival assay in the RN4220 background.

[00345] The survival ability of RN4220 and RN4220 *recA*$^-$ following exposure to Nor was then tested as described in Example 2 for *E. coli* strains. The *recA* null mutant and parental strains (RN4220 *recA*$^-$ and RN4220, respectively) were grown to early log phase in LB at 37°C with shaking. At the 0 time point Nor was added to the cultures to a final concentration of 0.5 µg/mL, which is just below the MIC for wild type cells (RN4220). Samples of the cells were collected and washed in PBS at 0, 30, 90 and 180 minutes time points. The number of viable cells was determined by plating the cells on LB from each time point and counting colonies. The CFU counts were normalized based on the time 0 time point inoculum for each culture (*i.e.*, the CFU count obtained from the 0 time point inoculum was taken to represent 100% survival) and the log %survival was plotted (Figure 5).

[00346] RN4220 *recA*$^-$ was observed to be highly sensitive to quinolone treatment and could not recover following a period of exposure to Nor. It was concluded that *recA* is essential for survival in the presence of quinolones (Figure 5). As in *E. coli, recA* deletion causes 10,000X increase in quinolone sensitivity compared to the parental strain after 3 hours of exposure to Nor.

[00347] Further details of the assay are as follows: Overnight mutant MG1655 or Staph cultures of clones were grown in 5 mL of LB with appropriate selective agents at 37°C with shaking. A morning culture was started in a 96 well plate with 16 µL of cells into 1.6 mL of LB and the appropriate plasmid selective agent. Cultures were grown at 37°C for approximately 3 hours to mid log phase A dilution series of Norfloxacin or Cipro was made. A 50 µg/mL stock of Norfloxacin/Cipro was diluted in a 2-fold dilution series to the final concentrations below. Serial dilutions were done with 100 µL of drug into 100 µL of H_2O. (The 50 µg/mL Norfloxacin/cipro stock was made by the dilution of 40 µL of 1.25 mg/mL stock in 960 ul of H_2O). 20 µL of each log phase culture was put into a well of one row of a 96 well plate containing 2 µL of LB and the appropriate selective agent. 4.5 µL of each drug concentration was arrayed into the 8 rows of a sterile 300 µL 96-well plate. 250 µL of inoculated culture was added to each column of the sterile 300 µL 96-well plate with drug. The final plate has a different clone in each column and a different Norfloxacin concentration in each row. Plates were grown overnight at 37°C with shaking. The OD600 of the plate was measured after 16 hours.

Survival assay in a quinolone resistant strain background

[00348] The Applicants then compared the ability of strains that carry a *recA*$^-$ allele in a quinolone resistant (*grlA542*) and nonresistant strain background to survive exposure to a lethal concentration of Nor (Trucksis M, Wolfson JS, Hooper DC. J Bacteriol. 1991 Sep;173(18):5854-60. A novel locus conferring fluoroquinolone resistance in Staphylococcus aureus). "Lethal" here refers to a concentration lethal to a wild type strain. The *grlA542* allele contains a mutation in the *grlA* locus (also called *parC*), resulting in a serine to phenylalanine change at position 80 in the GrlA subunit of *S. aureus* DNA topoisomerase IV that confers quinolone resistance on *S. aureus* strains that would otherwise be sensitive to quinolones (Fournier *et al.*, 2000). Cultures were grown to early log phase in LB at 37°C with shaking. Nor was added to the cultures to a final concentration of 2 µg/mL. Samples of the cells were collected and washed in PBS at 0, 120 (2 hr) and 180 (3 hr) min time points. The reduction in number of viable cells was determined by stamping identical 10-fold serial dilutions of the treated cells from each time point on LB as shown in Figure 6, where wt refers to wild type, recA^{mut} refers to the strain containing the recA null allele in an otherwise wild type strain background, grla542 refers to the strain containing the *grlA542* allele in an otherwise wild type strain background, and grlA542recA^{mut} refers to the strain

containing both the *recA* null allele and the *grlA542* allele. The MIC for strains wt, $recA^{mut}$, grla542 and $grlA542recA^{mut}$ were 0.78, 0.19, 12.5 and 1.56 μg/mL, respectively.

[00349] At time 0, the number of cells for the parental and $recA^-$ strains were almost the same, while the strain harboring the quinolone resistance mutation (*grlA542*) had more cells. After 2 and 3 hours of growth in presence of Nor, aliquots were taken, washed then serial dilution were plated onto LB containing plate without Nor. Colonies were observed only for the *grlA* mutant strain after 3 hours of treatment, while the *glrA- recA-* strain was barely able to form colonies (Figure 6). It was concluded that the *recA* mutant *S. aureus* strains are more sensitive to quinolone treatments in both wild type and quinolone resistant background.

[00350] Based on these results, inhibiting the expression and/or activity of *S. aureus* RecA is expected to increase the sensitivity of *S. aureus* to quinolones to which they already display sensitivity (*i.e.*, will reduce the MIC), thereby allowing the use of a lower dose of the drug for treating subjects suffering from or at risk of *S. aureus* infection. In addition, inhibiting the expression and/or activity of *S. aureus* RecA is expected to render *S. aureus* strains that have acquired resistance to quinolones (*e.g.*, due to mutation in one or more subunits of the bacterial gyrase or topoisomerase IV and/or due to increased copy number of a gene encoding one of these polypeptides) sensitive once again to these compounds.

[00351] The table below summarizes results of the screens and homology searches. The left column of the table lists quinolone potentiator target genes, *i.e.*, genes whose deletion resulted in significantly reduced growth or both significantly reduced growth and survival in either Nor, Cipro, or both. The remaining columns from the left indicate whether the mutant having a deletion of the indicated gene was able to grow or survive in the presence of sublethal or lethal concentrations of Nor. The fourth and fifth columns from the left indicate whether the mutant having a deletion of the indicated gene was able to grow or survive in the presence of a sublethal or lethal concentration of Cipro, respectively. In each case, "+" indicates that the strain failed to grow or survive. The column on the right indicates whether a homolog of the indicated gene was identified in *S. aureus*.

Gene	Norfloxacin		Ciprofloxacin		S. aureus
	Growth	Survival	Growth	Survival	homolog identified
recA	+	+	+	+	+
recB	+	+	+		+
recC	+	+			
recG	+	+	+		+
recN			+		+
ruvA	+	+			+
ruvB	+	+			+
ruvC	+	+			
uvrD	+	+			+
xerD			+		+
xseA			+		+
xseB			+		+
pinQ	+	+			
dnaT			+		
priA	+	+			+
polA			+		+
thyA			+		
acrA	+		+		
acrB			+		
tolC	+		+		
b1440	+				
ompC			+		
fabH	+	+			+
dksA	+				
fis	+	+	+		
dapF			+		
fliN	+	+			
jw5303	+	+			
yhfT	+	+			
yqgC	+	+			
ycjS	+	+			+
resA	+	+			
dapF	+	+	+		
ygoC	+				
rimK	+				
yicN			+		

Example 5: recA Null Mutation Reduces Bacterial Viability in Rats and Mice

[00352] The present Applicants studied the viability of *recA* null mutants in a rat model. *S. aureus* RN4220 and RN4220 *recA*$^-$ strains (see Example 3) were grown to logarithmic phase. Individual animals were inoculated with 10^2, 10^4 or 10^6 cells of either strain in a volume of 200 μL of PBS by intraperitoneal injection. A control animal was injected with PBS only. At the end of day 1, the animal infected with the highest inoculum of RN4220 was showing signs of illness while the 6 other animals were apparently unaffected. On day

4, the animals were sacrificed, and the spleens were collected and processed to quantify the progression of the infection using a colony formation assay. Briefly, a spleen tissue extract was made, which was then diluted and plated on antibiotic containing plates to allow colony formation. For the *recA*$^+$ strain, the numbers of colonies recovered were 8, 22, 104 for the animals inoculated with 10^2, 10^4 or 10^6 cells, respectively. For the *recA*$^-$ strain, the numbers of colonies recovered were 1, 0, 1 for the animals inoculated with 10^2, 10^4 or 10^6 cells, respectively. As expected, we recovered no colonies from the control animal injected with PBS only.

[00353] A larger experiment was then performed in mice to assess the effect of a *recA* null mutation in a Nor resistant strain background using the bacterial strains described in Example 4. Fifteen female CD-1 mice (20-22 g) were inoculated with about 5 x 10^7 CFU of bacterial strain grlA542 or grlA542 recA^{mut} (10^7 CFU) in 0.5 mL PBS via intraperitoneal injection. Survival of the mice was monitored at 24 and 48 hours after injection. By 48 hours after injection all of the mice inoculated with the recA$^-$ strain (grlA542recA^{mut}) survived, while all but one of the mice inoculated with the recA$^+$ strain (grlA542) perished. In other words, only 6.6% of the mice inoculated with the recA$^+$ strain survived while 100% of the mice inoculated with the recA- strain survived. These results show that *S. aureus* cells lacking a functional RecA are not able to survive in a rat and mice hosts, demonstrating that these models are useful for the identification and/or testing of potential inhibitors or antagonists of RecA.

Example 6: Development of a Cell-based Assay to Identify Inhibitors of RecA

[00354] As discussed above, the *recA* promoter is induced when RecA becomes activated as a result of exposure to DNA damaging agents. The present Applicants hypothesized that exposure to a quinolone may have a similar effect on the *recA* promoter and that this effect could be exploited to develop a cell-based assay for inhibitors of RecA. A low copy reporter plasmid was used in which the *recA* promoter controls transcription of the mRNA encoding green fluorescent protein (GFP). The plasmid, referred to herein as prec::GFP, has been previously described (Ronen, 2002). See also Kuang, 2004. prec::GFP contains 2821707-2821893 of the *E. coli* MG1655 genome (numbering based on the sequenced *E. coli* genome as reported in Blattner, F.R., et al., *Science*, 277, pp. 1453-1474, 1997) cloned upstream of a promoterless *GFPmut3* gene in a low copy pSC101 origin plasmid as described (Kalir, S., et al., *Science*, 292, pp. 2080-2083, 2001).

[00355] The prec::GFP plasmid was introduced into MG1655 and MG1655 *recA* null strains and measured the fluorescence emitted by GFP in the presence or absence of quinolone as compared with background fluorescence detected from cultures of MG1655 lacking the plasmid. Briefly cells of these three strains were grown to early log phase in LB at 37°C with shaking. Various amounts of Nor were added to 100 μL cultures of each strain to achieve final concentrations ranging from 0 μg/mL to 31.25 μg/mL. After 60 minutes, fluorescence due to GFP expression from the reporter plasmid was measured using a spectrophotometer.

[00356] As shown in Figure 7, fluorescence in a *recA*$^+$ wild type strain (red bars – middle bars in each group) was clearly detected while the fluorescence level in a *recA* null strain (yellow bars – right bars in each group) was close to the background level, *i.e.*, approximately the same as that of the wild type strain lacking the plasmid (blue bars – left bars in each group). Based on these results it would be expected that a compound that inhibits expression of RecA or inhibits any of a variety of functions of RecA will result in a decrease in GFP fluorescence (Figure 7) in cells containing the reporter plasmid.

Example 7: Development of an In Vitro Assay to Identify Inhibitors of RecA

[00357] As discussed above, RecA is a DNA-dependent ATPase. An assay was developed for RecA activity based on detection of the amount of ATP remaining in a reaction mixture following incubation of RecA protein, DNA, and ATP. 40-50 μg/mL of RecA (New England Biolabs) was combined with 20 μM ATP in a final volume of 50 μL PNK buffer 1x either with or without and 250 ng/μL of M13 DNA, and the reaction mixtures were incubated at 25°C for 30 minutes. 20 μM ATP-Gamma, a non-hydrolyzable form of ATP that inhibits RecA, was added to some of the reactions 10 minutes after the start of the incubation.

[00358] The assay measures the amount of ATPase (adenosine triphosphate (ATP) – adenosine diphosphate (ADP)) activity catalyzed by *E.Coli* RecA protein. In the assay, recA is incubated in reaction buffer with DNA, ATP and compounds of interest for a measured amount of time. The quantity of ATP remaining after the RecA reaction is quantitated using a subsequent luciferase assay. The amount of ATP remaining can be compared to inhibited and fully active control samples. Details of the assay are provided below.

[00359] **Materials.** Kingase glo plus, adenosine triphosphate (ATP), Adenosine 5'-O-(3-thiotriphosphate), and 384 well plates, white were obtained from Fisher; and M13mp18 Single-stranded DNA, RecA Protein and T4 Polynucleotide Kinase Reaction Buffer = PNK were obtained from New England Biolabs. PNK contained 70 mM Tris-CL pH 7.5, 10 mM $MgCl_2$ and 5 mM DTT.

[00360] **Assay Procedure.** Final reaction mixture contains: 0.5 μL of M13 single stranded DNA (250 μg/mL); 1.0 μL of 10X PHK buffer; 0.25 μL of RecA (2 mg/mL); 0.25 μL of 1 mM ATP; 0.25 μL of test compound in DMSO; and 12.0 μL of H_2O.

[00361] Test compounds are incubated at room temperature in the presence of RecA protein and DNA prior to addition of ATP. This can be achieved by the preparation of two mixes, as indicated below. Desirably, both a no-DNA and a DNA/DMSO-only (*i.e.*, no test compound) control were included.

Component	**Mix 1 (in μl)**	**ATP Mix (in μl)**
M13	0.5	0.0
10X PNK Buffer	1.0	0.25
RecA	0.25	0.0
1 mM ATP	0.0	0.25
1 mM ATP-G or Test compound	0.5	0.0
H_2O	7.75	2.0
Total each	10	2.5

[00362] The following steps are then performed: (1) Add 7.75 μL of mix 1 to the well of a 384 well plate; (2) Add 0.5 μL of the compound of interest; (3) Incubate at room temperature for 5 minutes; (4) Add 2.5 μL of the ATP mix. Incubate at room temperature for 30 minutes; (5) Add 12.5 μL of Promega Kinase glo plus; (6) Incubate at room temperature for 10 minutes or more; and (7) Measure luminescence of the reaction.

[00363] Figure 8 shows results of the assay when performed using four different reaction mixtures, as indicated on the figure beneath the bars showing luminescence (relative light units). Bars C and D illustrate the absence of RecA activity in reactions that contain ATP-gamma, which inhibits RecA. Controls in which no RecA was added were also performed. A comparison between bar A and bars C and D shows that RecA activity in the presence of DNA can be readily detected based on the reduced luminescence detected from reactions in which ATP has been consumed by active RecA. A comparison between bars A and B demonstrates that RecA activity is DNA dependent in this assay.

Example 8: Identification of Quinolone Potentiating Agents Using a RecA Inhibition Assay

[00364] Test compounds used in this screen are housed in wells of microwell plates in which 100 μg of each compound was resuspended in 50 μL of DMSO (2 μg/μL). The screen was performed using 384-well plates. A reaction mixture containing 2 mg/mL of RecA (New England Biolabs), 250 ng/ μL of M13 DNA, and 1 mM ATP in a final volume of 50 μL PNL buffer 1x was dispensed into each well. 5 μL of a different compound solution (10 μg of compound) was added to each well, one compound per well. The plate included 4 negative control wells in which no test compound was added to the reaction mixture, and 4 positive control wells, in which the reaction mixture also contained 1 nM ATP-gamma to maximally inhibit RecA and did not contain a test compound. The plates were incubated for 1 hour, following which 50 μL of the Promega Kinase-Glo™ Reagent (Promega) was added to each well. Luminescence was measured. Test compounds in wells in which the luminescence was lower than the average luminescence detected in the negative control wells were identified as RecA inhibitors. The extent of RecA inhibition by any particular test compound was assessed by comparing the luminescence with that detected in the positive control well, which represent 100% inhibition of RecA. Compounds that inhibit RecA ATPase activity by at least 80% were selected for further screening.

[00365] For each test compound, the difference between the OD600 for the well containing the test compound and Nor and the OD600 for the well containing the test compound but no Nor was calculated. If the OD600 for the well containing a particular test compound and Nor was significantly lower than the OD600 for the well containing the same test compound and not containing Nor, then the test compound was identified as a quinolone potentiating compound.

[00366] Compounds identified as RecA inhibitors using this assay were further tested to determine whether they are quinolone potentiating agents as described in Example 10.

Example 9: Identification of Quinolone Potentiating Agents Using a Cell-based RecA Reporter Assay

[00367] Test compounds used in this screen are housed in wells in microwell plates in which 100 μg of each compound is resuspended in 50 μL of DMSO (2 μg/μL). The screen was performed using 384-well plates. 50 μL of an early log phase culture of MG1655 containing the prec:GFP plasmid were dispensed into each well, and Nor was added to a final concentration of 10 μg/mL. As shown in Example 6, this concentration of Nor results in robust induction of the *recA* promoter. A different test compound was added to each well to a final concentration of 50 μg/mL. Each plate contained 4 control wells to which no test compound was added and 4 control wells to which 50 nM ATP-gamma was added to inhibit RecA. The wells were incubated at 37°C for 60 minutes, following which GFP fluorescence was detected. Compounds in wells that display decreased fluorescence relative to the control wells to which no test compound was added were identified as RecA inhibitors. The extent of RecA inhibition was assessed by comparison with fluorescence in the wells that contain ATP-gamma, which represents maximal inhibition of RecA (and thus approximately background levels of fluorescence).

Example 10: Demonstrating Quinolone Potentiation in E. coli

[00368] A growth assay was performed in order to demonstrate potentiation of norfloxacin by candidate potentiating agents, *e.g.*, compounds identified as RecA inhibitors using the assays described in Example 8 or 9. Samples of *E. coli* were cultured overnight in standard culture medium in the presence of Nor (control), and their growth was compared with that of samples of *E. coli* that have been cultured in the presence of both Nor (0.05 μg/mL) and a test compound (50 μg/mL) over the same time period and under otherwise identical conditions. The concentration of Nor used for the assay was not sufficient to kill and/or inhibit growth of *E. coli* by itself. The assay was performed in multiwell plates, *e.g.*, microtiter plates, in which each well contained a different test compound. Growth was quantified by measuring optical density (OD) using a Tecam spectrophotometer at a wavelength of 600 nm. Growth in the well containing both norfloxacin and a particular test compound was compared with growth in a well containing Nor (0.05 μg/mL) but not containing the test compound and was also compared with growth in a well containing the test compound but not containing Nor. Lack of growth or poor growth in the well containing both Nor and a particular test compound relative to growth in a well containing Nor but not containing a test compound indicates that the test compound is either a potentiator of Nor activity or has antibacterial activity by itself, or both. If, in addition, the presence of the test compound does not result in lack of growth or poor growth in the absence of Nor, then the test compound is a potentiator of Nor activity but apparently lacks antibacterial activity by itself, at least under the conditions of the assay, *i.e.*, the compound has quinolone-dependent antibacterial activity.

[00369] Compounds that were determined to have quinolone-dependent antibacterial activity were tested using the same method at lower concentrations.

[00370] Compounds identified as quinolone potentiating compounds using this screen were tested to determine whether their potentiating activity is specific to quinolones by examining their ability to inhibit bacterial growth in the presence of sublethal concentrations of other antibiotics, *e.g.*, macrolides, aminoglycosides, penicillins, cephalosporins, etc. Compounds that fail to inhibit bacterial growth in the presence of sublethal concentrations of other antibiotics were identified as being specific quinolone potentiating agents.

[00371] Compounds identified as quinolone potentiating agents were tested to determine whether they affected the growth of a variety of cell types at a range of concentrations. Compounds that have little or no effect on mammalian cell growth at concentrations at which they display quinolone potentiation were identified as being particularly promising for use in a therapeutic context.

Example 11: Demonstrating Quinolone Potentiation in Staphylococcus aureus.

[00372] Example 10 was repeated except that *Staphylococcus aureus*, a gram-positive coccus, was used instead of *E. coli*, in order to demonstrate that test compounds potentiate quinolone activity against a broad spectrum of microorganisms.

Example 12 : Demonstrating Quinolone Potentiation in Pseudomonas aeruginosa.

[00373] *Pseudomonas aeruginosa* is an opportunistic bacterial pathogen that, for example, causes chronic lung infections in cystic fibrosis patients and is a major source of nosocomial infections. Example 10 was repeated except that *Pseudomonas aeruginosa*, was used instead of *E. coli*, in order to demonstrate that test compounds potentiate quinolone activity against this important pathogen.

Example 13: Demonstrating Quinolone Potentiation in Ciprofloxacin-resistant Pseudomonas aeruginosa.

[00374] Resistance of *P. aeruginosa* to quinolones is becoming an increasing problem. Example 10 was repeated except that a clinical isolate of ciprofloxacin-resistant *Pseudomonas aeruginosa*, was used. The strain has a MIC of about 16 μg/mL (determined as described in Lomholt, JA and Kilian, M. *Br. J. Ophthalmol.*, 87: 1238-1240, 2003). The strain exhibits essentially no growth inhibition when cultured *in vitro* in medium containing

.25 μg/mL ciprofloxacin. The bacteria were also cultured in medium containing .25 μg/mL ciprofloxacin and 1 μg/mL of a quinolone potentiating agent identified as described above. The bacteria were unable to grow, demonstrating that the combination of a quinolone and a quinolone potentiating agent is effective against bacteria that are resistant to the quinolone alone.

Example 14: Synergistic Effect of a Quinolone and a Quinolone Potentiating Agent Allows Reduction in Quinolone Dose

[00375] Administration of certain quinolone antibiotics, *e.g.*, gatifloxacin, has been shown to result in a prolongation of the QT interval on the electrocardiogram, which can lead to dangerous cardiac arrhythmias such as *torsades de pointes*. Certain of these agents are highly effective antibiotics. However, concerns regarding their potential to cause arrhythmias when administered at clinically effective doses has led to termination of development of certain of these agents or their withdrawal from the market. It would be desirable to be able to administer these agents at a lower dose that would not have proarrhythmic consequences while still providing a clinically useful antibacterial effect.

[00376] *E. coli* are cultured at a concentration of gatifloxacin below its MIC and their growth is assessed as described in Example 1. Bacterial growth is not significantly inhibited. However, when bacteria are cultured in the presence of a quinolone potentiating agent and the same sub-MIC concentration of gatifloxacin, they fail to show significant growth, demonstrating that the combination of a quinolone potentiating agent and a quinolone that is too toxic for routine clinical use allows the use of a reduced amount of the quinolone.

Example 15: RecA Activity Assay - DNA Binding Assay

[00377] This assay measures RecA DNA binding activity, and can be used to identify or characterize RecA inhibitors according to the present invention.

[00378] The plasmid pUC19 contains 5 HpyCH4 IV sites, including one at position 374. A 60 mer oligonucleotide was designed having complementarity to the region in pUC19 that centers around the HpyCH4 IV site at position 374. The following reaction components were combined in 40 μL RecA Reaction Buffer:1 μg of pUC19; 0.18 μg of 60 mer; 0.3 mM ATP γ–S; and 4 μg of RecA, and were incubated at 37°C for 10 minutes, so that a stable triple helix was formed. Unprotected sites were methylated using 8 units of SSS I

supplemented with 160 μM SAM for 10 minutes at 37°C. The reaction was stopped and the triple helix was disrupted by incubation at 65°C for 15 minutes. The reaction was cooled and 10 units of HpyCH4 IV were added. Digestion was allowed to proceed at 37°C for 20 minutes. Greater than 90% of the product was single cut pUC19. Thus, this assay can be used to identify and/or characterize RecA inhibitors because, if they interfere with RecA DNA binding, then they will ultimately reduce the amount of single cut product observed.

Example 16: RecA Activity Assay - Biochemical Co-Protease Assay

[00379] In this assay, which is based on an assay developed by Dutreix *et al.*, J. Bacteriol., 1989, 171: 2415), the LexA coding sequence will be fused to a HIS tag (Qiagen), and expressed from the pQE vector under control of the T5 promoter. This vector allows for expression of C-terminally 6xHis-tagged proteins. The *lexA* ORF will be created by introducing an NcoI restriction site at the ATG codon of the insert by PCR. Identity of the insert will be confirmed by DNA sequencing. A test expression experiment will be performed and the integrity of the LexA protein will be checked using an anti-HIS antibody as described in the Qiagen kit. The QIA*express* detection reagents include high-affinity, high-specificity monoclonal Anti·His antibodies and antibody conjugates, and Ni-NTA conjugates.

[00380] The pQE-lexA plasmid will be transformed into a *recA*+ and *recA*- strains to test the assay genetically. In a *recA*+ background, LexA protein is expected to be seen intact unless DNA damage is induced, *e.g.*, by exposure to an appropriate antibiotic agent (*e.g.*, a quinolone). If DNA damage is induced, either a cleaved (shorter) LexA or no LexA protein is expected to be observed. LexA protein can be detected using a Western blot assay. In a *recA*- background, LexA is expected to be seen intact, whether or not DNA damage is induced.

[00381] Once the assay is validated, it can be used to assess the ability of RecA inhibitors to interfere with the RecA co-protease activity. It is expected that addition of RecA inhibitors, prior to or during DNA damage (*i.e.*, exposure to quinolone antibiotic agent), will decrease the amount of LexA degradation, and will increase the amount of full-length LexA.

[00382] Typically, assay reactions will be performed so that 50 μg/ml of test compound is utilized in the presence of 300 ng/ml of Cipro. This amount of Cipro is enough to induce the RecA response but is not enough to kill the cells.

[00383] Typically, 2 ml of *E. coli* cells containing pQE-lexA will be grown to an OD_{600} of 0.5, then exposed to Cipro or to Cipro + test compound for 30 – 60 minutes. The cells will be harvested and disrupted; the protein extract will be applied on to the Ni-NTA conjugate column, aliquots will be run onto a SDS-PAGE, and LexA detection will be done using the anti-HIS antibodies. Compounds for which the LexA protein remains intact likely due to RecA inhibition will be further examined.

Example 17: Cell-based Survival and Cell Growth Assays

[00384] ***Cell-based Survival Assay.*** This assay measures the number of cells that are able to form colonies (*i.e.*, the number of colony forming units, CFU) after a culture has been treated with a particular antibiotic agent (*e.g.*, norfloxacin or cipro). Typically, the antibiotic agent is applied to an early log phase culture at a concentration that is above the minimum inhibitory concentration ("MIC"; the minimum concentration that will inhibit growth) for the relevant strain under the relevant growth conditions. For example, the antibiotic agent may be applied at a level that is a multiple of the MIC, often 4x or 8x MIC. Samples of the cells are collected at various time points after addition of antibiotic agent (*e.g.*, 0, 18, and 26 hours). The samples are washed and plated (*e.g.*, in serial dilutions), typically on rich media, so that the number of CFU can be counted. CFU are then normalized to the time 0 time point, and can be plotted, for example, on a log graph.

[00385] Such a cell-based survival assay can be used to evaluate a test agent alone or in combination with another antibiotic agent. Often, a control assay is done with a known antibiotic agent, and in the absence of any test agent. Such a control assay can be compared with test agent alone and/or with known antibiotic + test agent.

[00386] ***Cell Growth Assay.*** This assay measures the ability of cells to grow in the presence of a sublethal dose (*i.e.*, a dose below the MIC) of an antibiotic agent (or test agent, or combination thereof). In general, cells are grown, typically to saturation, and are then diluted and inoculated onto rich medium and onto medium containing a sublethal dose of antibiotic agent. Plates are then grown overnight, and OD_{600} is measured after overnight growth, as compared with blank plate. If desired, a test agent can be added to the plate, either alone or in combination with the known antibiotic agent, and the ratio of OD_{600} in the presence of the test agent to OD_{600} in the absence of the test agent, can be determined. Additional ratios that can be useful as controls include, for example, OD_{600} in the presence vs absence of antibiotic agent; OD_{600} in the absence of any agent *vs* in the presence of both antibiotic agent and test agent; etc.

Example 18: Mutation/Resistance Assay

[00387] This assay measures the rate at which mutant cells arise in the presence of an antibiotic agent. Cells are grown in the presence of antibiotic agent at a concentration above (generally well above, *e.g.*, 4x or 8x) its MIC, and further in the presence or absence of a test agent (that may inhibit or enhance mutation).

[00388] Every 24 hours, an aliquot is removed from the culture and is diluted so that the antibiotic agent is no longer present at a concentration above its MIC (*e.g.*, so that the antibiotic agent is present at 0.5x MIC or less). These aliquoted samples are then assayed to determine whether they can grow in the presence of the antibiotic agent. Specifically, when growth is observed in the presence of antibiotic agent at a concentration that is 4x MIC, then resistance is said to have developed. Test compounds can therefore be assayed to assess whether or not they can reduce the incidence of resistance (*e.g.*, by inhibiting mutation).

Example 19: In vivo Assay of RecA Inhibitor Activity in Rats

[00389] An *in vivo* system has been developed for the identification and/or characterization of RecA inhibitors in rats. Specifically, the present Applicants have determined that infectious disease can be established in rats by intraperitoneal injection of an appropriate number of bacterial cells. They have further demonstrated that injection of the same number of otherwise identical cells lacking RecA (*i.e.*, *recA-* mutant cells) does not establish infection. Furthermore, by isolating spleens from the infected rats and plating the colony forming units from within those spleens, they have established that *recA-* cells *both* (1) are avirulent; (2) and do not survive or grow in the animal.

[00390] For example, each of 7 rats were injected with one of the following agents in 200 µl PBS: no cells; 10^2, 10^4, or 10^6 CFU *Staphylococcus aureus* RN4220 (RecA+); 10^2, 10^4, or 10^6 CFU *Staphylococcus aureus* RN4220 *recA-*. On day 1, the rat that received 10^6 CFU RN4220 (RecA+) was sick. On day 4, all rats were sacrificed and their spleens were recovered and processed. Cells were plated, and CFU present in the spleens were counted, with the following results:

Pathogen		**CFU Recovered from Spleen**
No cells	0 cells	0
RecA+ cells	10^2 cells	8
	10^4 cells	22
	10^6 cells	104
recA- cells	10^2 cells	1
	10^4 cells	0
	10^6 cells	1

[00391] These results demonstrate that inactivation of RecA in bacterial cells reduces their ability to cause sickness, and also reduces the number of CFU they can establish in rats. This system therefore can be used to identify and/or characterize inventive RecA inhibitors. The *recA* deletion strain can desirably be used as a positive control.

Example 20: In Vivo Assay of RecA Inhibitor Activity in Mice

[00392] This Example describes uses an *in vivo* system for the identification and/or characterization of RecA inhibitors in mice. Specifically, the present Applicants have determined that infectious disease can be established in mice, and indeed can kill the mice, by intraperitoneal injection of an appropriate number of bacterial cells. They have further demonstrated that all mice survive when the cells that are injected (same number of otherwise identical cells) lack RecA (*i.e.*, are *recA-* mutant cells).

[00393] For example, each of 30 female CD-1 mice were injected with one of the following:> 5 x 10^7 CFU *Staphylococcus aureus* grlA542 (RecA+); or > 5 x 10^7 CFU *Staphylococcus aureus* grlA542recA^{mut} (*recA-*) in 0.5 ml PBS (see Fournier *et al.*, Antimicrob. Agents Chemother., 2000, 44: 2160). The number of dead animals was assessed at 24 hours and at 48 hours, with the following results:

Pathogen	**Number of Surviving Mice**
At 48 hours:	
RecA+ cells	1/15 (6.6%)
recA- cells	15/15 (100%)

[00394] These results indicate that loss of RecA function renders pathogens non-lethal in mice. These results also demonstrate that this system can be used to identify and/or characterize inventive RecA inhibitors (which, when administered prior to or during infection, should reduce lethality). The *recA* deletion strain can desirably be used as a positive control.

Example 21: In Vivo Assay of RecA Inhibitor Activity in Immunocompromised Mice

[00395] This Example describes an *in vivo* system for the identification and/or characterization of RecA inhibitors in immunocompromised mice. The experiment was similar to that described above in Example 20 except that, 4 days prior to infection, mice were rendered immunocompromised by treatment with 150 mg/kg cyclophosphamide in 0.5 ml sterile water. One day prior to infection, the mice were treated again with 100 mg/kg cyclophosphamide, again in 0. ml sterile water. Mice were then infected with RecA+ or *recA- S. aureus* as follows: 10^4, 10^5, or 10^6 CFU *Staphylococcus aureus* grlA542 (RecA+); or 10^4, 10^5, or 10^6 CFU *Staphylococcus aureus* grlA542recA^{mut} (*recA-*) in 0.5 ml PBS (see Fournier *et al.*, Antimicrob. Agents Chemother., 2000, 44: 2160). The number of dead animals was assessed at 24 hours, with the following results:

Pathogen		**Number of Surviving Mice**
RecA+ cells	10^4 cells	2 of 5
	10^5 cells	1 of 5
	10^6 cells	0 of 5
recA- cells	10^4 cells	5 of 5
	10^5 cells	2 of 5
	10^6 cells	2 of 5

[00396] These results indicate that loss of RecA function dramatically reduces pathogenicity even in immunocompromised mice. These results also demonstrate that this system can be used to identify and/or characterize inventive RecA inhibitors. The *recA* deletion strain can desirably be used as a positive control.

Example 22: Serum Binding Assay

[00397] The present Applicants have found that it can be desirable to assess the activity of RecA inhibitors in the presence of serum as an indicator of whether they are likely to retain activity when administered *in vivo*. Any one or more of the activity/characterization assays described herein can be performed so that a test agent/RecA inhibitor is exposed to serum for a period of time before or during assessment of its activity.

Example 23: Identification of RecA Inhibitors Using In Vitro Luciferase Assay

[00398] More than 2000 compounds were tested for their ability to inhibit RecA ATPase activity in the luciferase assay described in Example 7. Tested compounds were obtained from Boston University (1205 compounds from CMLD diversity library, available from Dr. Scott Schauss), IndoFine (66 compounds based on flavone structure), ChemdiV (48 compounds known to be ATPase inhibitors and 720 compounds structurally related to CB101 (described, for example, in USSN Serial Number 60/662,038), and Asinex. Five (5) tested compounds were bisflavones and 28 were predicted, based on structural modeling (see, for example, Example 31) to dock with Rec A. Many of the compounds are lipophilic, natural-product-like compounds. Others are flavones, bisflavones, flavonoids, coumarins, and/or heterocyclic compounds.

[00399] Of these compounds, 1 was found to have an IC_{50} in this screen of below 100 μg/ml; 11 were found to have an IC_{50} below 50 μg/ml, 9 were found to have an IC_{50} below 15 μg/ml; 2 were found to have an IC_{50} below 10 μg/ml; 5 were found to have an IC_{50} below 5 μg/ml; 3 were found to have an IC_{50} below 3 μg/ml; and 1 was found to have an IC_{50} below 1 μg/ml. Structures of the 14 compounds that inhibited RecA ATPase activity by more than 50% are presented in Figure 14. Percent inhibition is calculated according to the following equation:

$$\frac{(\text{RLU in presence of compound}) - (\text{RLU in DMSO})}{(\text{RLU in absence of DNA}) - (\text{RLU in DMSO}).}$$

A value of "-1" indicates higher than 50 μg/ml, therefore undetermined.

[00400] Figure 14 presents representative structures of 14 particularly active compounds, including amentoflavone, apigenin, apigenin-7-O-glucoside, fitsetin, hinokiflavone, 6-hydroxy-apigenin, isorhamnetin, kaempferol, maclurin, morin, quercetagetin, quercetin dihydrate, 3,7,4'-trihydroxyflavone, fisetin, and theaflavin. Of these, hinokiflavone shows the lowest IC_{50} (<1 μg/ml).

Example 24: Confirming RecA ATPase Inhibition Activity of Hinokiflavone Using In Vitro Luciferase Assay

[00401] In order to confirm the result obtained in the screen, the present Applicants retested hinokiflavone in the luciferase assay described in Example 15. Hinokiflavone was added at time t = 0 or at time t = 30 minutes, and several different concentrations were tested. Figure 17 shows that hinokiflavone completely inhibited RecA ATPase activity at a concentration of 2.9 μM.

Example 25: RecA Inhibitors in Survival Assay

[00402] Several RecA inhibitors that showed an ability to inhibit RecA ATPase activity in the luciferase assay of Example 23 were tested for their ability to potentiate ciprofloxacin antibiotic activity in a survival assay as described in Example 17. In particular, *S. aureus* strain S3 (ISP 794) was grown in the presence of 1 μg/mL ciprofloxacin and in the presence or absence of 50 μg/mL of each compound for 16 hours.

[00403] Figure 18 shows a survival assay plate of 11 different RecA inhibitors. As can be seen, only one of the compounds (hinokiflavone) potentiated ciprofloxacin activity. Indeed, each of the other compounds had a protective effect, allowing more cells to survive ciprofloxacin treatment than were able to do so in the absence of the RecA inhibitor. Without wishing to be bound by an particular theory, we note that such a protective effect might be expected, for example, if these compounds activate DNA repair, inhibit DNA gyrase, or generally have after effects in the cells.

Example 26: Hinokiflavone Activity in Survival Assay

[00404] *S. aureus* strain S3 was used in a survival assay as described in Example 17. The strain was grown in the presence of the following agents: DMSO alone; DMSO + 1 μg/mL ciprofloxacin; DMSO + 25 μg/mL hinokiflavone; or DMSO + 1 μg/mL ciprofloxacin + 25 μg/mL hinokiflavone.

[00405] Figure 19 shows a photo of the 16 hour growth time point. As can be seen, hinokiflavone strongly potentiates ciprofloxacin antibiotic activity. Indeed, approximately 100 fold (2 logs) fewer CFUs are observed in the presence of hinokiflavone as compared with in its absence. Although it is not immediately apparent from Figure 19, several repeats of this experiment have also revealed modest antibiotic activity of hinokiflavone alone (*i.e.*, in the absence of ciprofloxacin), particularly at high concentrations.

[00406] Similar results were achieved when the experiment was performed with a different *S. aureus* strain (S7) that is mildly resistant to ciprofloxacin. Specifically, hinokiflavone potentiated cirpofloxacin activity against this strain.

[00407] Hinokiflavone was not observed to have antibiotic activity, or an ability to potentiate ciprofloxacin when the experiment was performed with an *E. coli* strain rather than an *S. aureus* strain. Given that *E. coli* are gram negative whereas *S. aureus* are gram positive, it is possible that this different result represents differential ability of the compound

to enter the cells (*e.g.*, due to the stronger efflux pump and/or second lipid membrane found in gram negative cells), although in our hands, simple measures such as using a strain with a defective an efflux pump more permeable membranes were insufficient to reveal activity. Those of ordinary skill in the art appreciate that additional measures are commonly required to ensure effectiveness of antibiotic compounds in the treatment of gram negative infections as compared with gram positive infections.

Example 27: Hinokiflavone Activity in Growth Assay

[00408] The present Applicants have found that concentrations of hinokiflavone that potentiate the antibiotic activity of ciprofloxacin in a survival assay (*i.e.*, that potentiate cell killing by cirpofloxacin) do not increase the MIC of ciprofloxacin in a growth assay.

Example 28: Hinokiflavone Targets RecA; Does Not Potentiate Ciprofloxacin in the Absence of RecA

[00409] *S. aureus* strains S3 (RecA+) and S4 (*recA*-) were used in a survival assay as described in Example 17. The strain was grown in the presence of the following agents: 1 μg/mL ciprofloxacin (in DMSO) or 1 μg/mL ciprofloxacin + 25 μg/ml hinokiflavone (in DMSO). Figure 20 is a graph showing that, in the absence of RecA (*i.e.*, in the *recA*- strain S4), hinokiflavone does not potentiate the activity of ciprofloxacin. This finding confirms RecA as the target of hinokiflavone.

[00410] The data presented in Figure 20, in fact, show a modest *protective* effect of hinokiflavone in the absence of RecA. That is, in the *recA*- strain S4, *more* cells survived in the presence of both ciprofloxacin and hinokiflavone than did in the presence of ciprofloxacin alone. One potential explanation for this finding is that the culture grown in the presence of both agents had fewer CFU at time point t = 0 than did the culture grown in the presence of ciprofloxacin alone, although numbers were normalized for initial cell count. Alternatively or additionally, it is possible that the RecA+ and *recA*- strains have different growth rates. Thus, data points on the left hand sides of the graphs (*e.g.*, up to about the 0.05 μg/ml ciprofloxacin data points) may well be more reliable than other data points.

Example 29: Stability of Hinokiflavone in Serum

[00411] The present Applicants have assessed the ability of hinokiflavone to potentiate ciprofloxacin's antibiotic activity in a survival assay in LB and in LB with 10% mouse serum, and found that hinokiflavone retained activity in the presence of mouse serum.

Example 30: Hinokiflavone Reduces the Incidence of Resistance to Ciprofloxacin

[00412] The present Applicants have found that the presence of hinokiflavone can reduce the ability of *S. aureus* cells to develop resistance to the fluoroquinolone antibiotic known as ciprofloxacin. Specifically, *S. aureus* cells were grown in the presence of 4x MIC of ciprofloxacin, and further in the presence or absence of hinokiflavone (25 μg/mL) .

[00413] Aliquots were removed from the cultures every 24 hours, and were diluted such that the concentration of ciprofloxacin present in the culture was reduced to 0.5x MIC. These aliquoted samples were then assayed to determine whether they can grow in the presence of ciprofloxacin. Specifically, when growth is observed in the presence of ciprofloxacin at a concentration that is 4x MIC, then resistance is said to have developed. In the present experiment, the Applicants counted the number of wells in which growth was observed in the presence of ciprofloxacin at a concentration that is 4x MIC in order to assess the extent of resistance developed in our original culture. The following results were obtained:

Number of Days Culture was Grown	**Number of Wells in which Growth Observed in Presence of Ciprofloxacin at 4x MIC**	
	- Hinokiflavone	**+ Hinokiflavone**
0	0	0
1	1	0
5	2	0
6	2	0
7	3	3
10	11	4

[00414] These results demonstrate that the presence of hinokiflavone decreases the incidence of resistance to ciprofloxacin. Similar results would be expected to be obtained with other antibiotic agents, particularly with other quinolones and/or aminoglycosides.

Example 31: Defining a Binding Site for Hinokiflavone on RecA

[00415] The structure of RecA protein, in some cases bound to a substrate and/or to DNA, has been reported. For example, structures have been established for RecA from bacteria such as *E.* coli (Rossbach *et al.*, BMC Struct. Biol., 2005, 20: 7; Story and Steitz, Nature, 1992, 355: 374; Story *et al.*, Nature, 1992, 355: 37, Van Look *et al.*, J. Mol. Biol., 2003, 333: 35; Xing and Bell, Biochemistry, 2004, 43: 1612; Yu and Edelman, Nat. Struct. Biol., 1997, 4: 101), *Mycobacterium sp.* (Datta *et al.*, J. Bacteriol., 2003, 185: 4280; Datta *et al.*, Nuc. Acid Res., 2000, 28: 4964), *Proteus mirabilis* (Weber and Steitz, J. Mol. Biol., 1986, 188: 109), and for RecA from *Archea* (Ariza *et al.*, Nuc. Acids Res., 2005, 33: 165; Wu *et al.*, Mol. Cell, 2004, 15: 423). The structure of the human RecA homolog, known as RAD51, has also been determined (Conway *et al.*, Nat. Struc. Mol. Biol., 2004, 11: 791; Wu *et al.*, J. Biol. Chem., 2005, 280: 722).

[00416] Binding interactions between particular compounds and a site or sites on a target molecule can be determined by molecular modeling programs that are known to those of ordinary skill in the art. These molecular modeling programs include, for example, QUANTA (Accelrys Inc., San Diego, CA) and the SYBYL suite of computational informatics software (Tripos Associates, Inc., St. Louis, Mo.).

[00417] Using the known crystal structure for *E. coli* RecA, the present Applicants have predicted binding sites for hinokiflavone and quercetin by using docking software to predict optimal interactions.

[00418] This strategy correctly predicted the ATP binding site, as well as various other known functional sites and ion binding sites. Moreover, it defined two novel binding sites (see Figures 21-28), one of which involves R85, F270, Y271, K310, and R32. This site is situated on the outward surface of RecA (see Figures 26 and 27), and is found in all species investigated (see Figure 28). In particular, the site is found in both gram positive and gram negative RecA proteins. Inhibitors binding such a site can act as broad-spectrum antibiotics, useful in the treatment of infections caused by both gram positive and gram negative organisms.

[00419] Work to prepare a co-crystal of RecA and hinokiflavone is in progress.

Example 33: Predicting Additional RecA Inhibitors

[00420] Having identified hinokiflavone as a particularly potent RecA inhibitor for use in accordance with the present invention, the present Applicants have assembled a collection of related compounds likely to share some or all of hinokiflavone's activities. For example, a variety of different flavones are known to inhibit helicases (see, for example, Xu *et al.*, Nuc. Acids Res., 2001, 29: 5058). Bisflavones are of particular interest, given their structural relationship to hinokiflavone.

[00421] Figure 15 presents a variety of exemplary potential RecA inhibitors according to the present invention that show significant structural similarity to hinokiflavone.

[00422] The present Applicants also considered the likelihood that compounds sharing electronic characteristics with hinokiflavone may well have similar ability to interact with RecA. Figure 16 presents a variety of exemplary potential RecA inhibitors according to the present invention that show significant electronic similarity to hinokiflavone.

Example 34: Identification of Mutations that Potentiate Aminoglycosides

[00423] Each knockout strain was first pregrown in 50 μg/mL of Kanamycin (an aminoglycoside antibiotic) in LB broth, then regrown in LB broth without Kanamycin. A sample of these regrown cultures was then diluted 1/250 and stamped onto an LB agar plate. The remaining culture was then treated with 4 μg/mL of Gentamicin for 1.5 hours. After treatment, a second sample was taken and diluted 1/250 in dH_2O before being stamped onto an agar plate. After 24 hours of growth at 37°C the size and density of each KO strain to the untreated intensity value was then calculated and ranked in order to target gene knockouts which cause the most significant increases in antibiotic susceptibility.

[00424] Target genes that were identified using the method described above are presented on Figure 29. These target genes include *aceE, araJ, aspA, atpA, atpF, atpH, brnQ, cydB, degP, dnaT, fepD, folP, guaB, Hfq, iscS, JW5360, lipA, lpdA, nuoB, nuoJ, nuoL, osmB, pdxH, pnuC, priA, priB, rbfA, recA, rfaD, rpmJ, rpoD, resA, ruvC, sdhC, seqA, setA, sucB, surA, tar, tatE, tauB, trmU, ubiH, ubiX, xerC, yaaU, ycfM, yejO, ygfZ, ygiH, yheL, yheM, yheN, yicG, yjdL, yjeT, yjiO,* and *yjjY.*

[00425] It is of interest to note that recA is one of the identified target genes. In a survival assay similar to that described in Examples 4, the present Applications have shown that the RecA deletion increases the sensitivity of S. aureus to aminoglycosides (Gentamicin). The results of this assay are reported in Figure 30.

Example 35: Mefloquine Potentiates the Aminoglycoside Gentamicin

[00426] Several aminoglycoside (gentamicin) potentiator target genes identified in Example 34 are ATP synthases. Mefloquine (a 4-quinolinemethanol derivative), which is a known inhibitor of ATP synthase, was found to potentiate Gentamicin. In strain E1, Mefloquine was found to reduce the MBC (minimal bacterial concentration) and MIC

(minimal inhibitory concentration)of gentamicin by 6 fold and 2 fold, respectively (see Figure 31), when used at a concentration of 100 μg/mL and 500 μg/mL, respectively.

Example 36: Identification of Mutations that Potentiate Antibiotic Cyclic Peptides

[00427] Identification of antibiotic cyclic peptide potentiator target genes were identified using the same method as that described in Example 34 using 20 μg/mL of Colistin in place of 4 μg/mL of Gentamicin

[00428] Target genes that were identified in the case of colistin using the method described above are presented on Figure 32. These target genes include *agaAk, atpA, atpF, atpH, bglF, cysE, cysI, fepC, fepD, frvR, guaA, guaB, hofF, hsdS, iscS, JW4016, JW5075, JW5227, JW5257, JW5360, kdgK, lipA, lysA, malG, mbhA, mdoG, Nei, nmpC, nudH, pdxH, phnB, phnL, phnO, pnuC, potE, pshM, ptsA, rhaT, rpiA, resA, Sbp, speA, sucB, sugE, tdcE, tdcG, tolC, trxA, ubiE, ubiH, ubiX, Xni, ybbY, ycfM, ydeJ, yeeY, yfeT, ygaA, ygfZ, yhdX, yheL, yheM, yiaY, yidK, yihV, yjbN, yjcR, yjcZ, ynjD, yqeC, yqiH,* and *yrfA.*

Example 37: Mefloquine Potentiates Colistin

[00429] Several colistin potentiator target genes identified in Example 36 are ATP synthases. Mefloquine, a known inhibitor of ATP synthase which was shown above to potentiate Gentamicin, was also found to potentiate Colistin (see Figure 33).

Example 38: Comparison of Target Genes Identified for Quinolones, Aminoglycosides and Antibiotic Cyclic Peptides

[00430] Quinolone potentiator target genes identified according to the present invention using ciproflaxin include *dapF, fabH, fis, fliN, jw5303, priA, recA, recB, recC, recG, resA, ruvA, ruvB, ruvC, ruvC, uvrD, ycjS,ydfL, yhfT,* and *yqgC.*

[00431] Aminoglycoside potentiator target genes identified according to the present invention using gentamicin include *aceE, araJ, aspA, atpA, atpF, atpH, brnQ, cydB, degP, dnaT, fepD, folP, guaB, Hfq, iscS, JW5360, lipA, lpdA, nuoB, nuoJ, nuoL, osmB, pdxH, pnuC, priA, priB, rbfA, recA, rfaD, rpmJ, rpoD, resA, ruvC, sdhC, seqA, setA, sucB, surA, tar, tatE, tauB, trmU, ubiH, ubiX, xerC, yaaU, ycfM, yejO, ygfZ, ygiH, yheL, yheM, yheN, yicG, yjdL, yjeT, yjiO,* and *yjjY.*

[00432] Antibiotic cyclic peptide potentiator target genes identified according to the present invention using colistin include *agaAk, atpA, atpF, atpH, bglF, cysE, cysI, fepC, fepD, frvR, guaA, guaB, hofF, hsdS, iscS, JW4016, JW5075, JW5227, JW5257, JW5360,* *kdgK, lipA, lysA, malG, mbhA, mdoG, Nei, nmpC, nudH, pdxH, phnB, phnL, phnO, pnuC, potE, pshM, ptsA, rhaT, rpiA, resA, Sbp, speA, sucB, sugE, tdcE, tdcG, tolC, trxA, ubiE, ubiH, ubiX, Xni, ybbY, ycfM, ydeJ, yeeY, yfeT, ygaA, ygfZ, yhdX, yheL, yheM, yiaY, yidK, yihV, yjbN, yjcR, yjcZ, ynjD, yqeC, yqiH,* and *yrfA*.

[00433] Overlapping target genes in the three classes of antibiotics are shown on Figure 34. Target genes common to the classes of aminoglycosides and quinolones are *priA, recA, resA,* and *ruvC*. Target genes common to the classes of aminoglycosides and cyclic peptides are *sucB, ubiH and ubiX, ycfM, ygfZ, yheL and yheM, atpA, atpF, and atpH, iscS, JW5360, guaB, lipA, pdxH, resA* and *pnuC*. *ResA* was found to be the only target gene common to all three classes of antibiotics.

Example 39: Potentiating Cidal Antibiotics via RecA Inhibition

[00434] The present Applicants have shown that the three major classes of bactericidal antibiotics (*i.e.*, antibiotics that act by inhibition of DNA replication and repair, inhibition of protein synthesis, and inhibition of cell-wall turnover) stimulate the production of highly deleterious hydroxyl radicals in gram-negative and gram-positive bacteria, which ultimately contribute to cell death (see M.A. Kohanski *et al.*, "A common mechanism of cellular death induced by bactericidal antibiotics", which is incorporated herein by reference in its entirety, including the supplementary information section). The results obtained suggest that all three major classes of bactericidal drugs can be potentiated by targeting bacterial systems that remediate hydroxyl radical damage, including proteins involved in triggering the DNA damage response, *e.g.*, RecA.

Methods

[00435] **Media and Antibiotics.** All experiments were done in Luria-Bertani (LB) medium (Fisher Scientific). The following bactericidal drugs were used: the cell wall synthesis inhibitors, ampicillin (Fisher Scientific) for *E. coli* and vancomycin (TEKnova, Hollister, CA) for *S. aureus*; the protein synthesis inhibitor kanamycin (Fisher Scientific), and the DNA gyrase inhibitor norfloxacin (Sigma). Ampicillin was used at concentrations of 5 and 15 μg/mL. Vancomycin was used at concentrations of 1 and 5 μg/mL. Kanamycin was used at concentrations of 5 and 25 μg/mL. Norfloxacin was used at concentrations of 25 and 250 ng/mL for experiments with *E. coli*, and a concentration of 4 μg/mL for experiments with S.aureus.

[00436] The following bacteriostatic drugs were used: the RNA polymerase inhibitor, rifamycin SV (MP Biomedicals, Solon, OH), and the protein synthesis inhibitors chloramphenical (Acros Organics, Geel, Belgium), tetracycline (MP Biomedicals), erythromycin (Sigma) and stectinomycin (MP Biomedicals). Rifamycin SV was used at concentrations of 300 and 500 mg/mL. Chloramphenical was used at concentrations of 7 and 15 μg/mL for the *E. coli* experiments and 20 mg/mL for the *S. aureus* experiments. Tetracycline was used at concentrations of 4 and 10 μg/mL. Erythromycin was used at concentrations of 200 and 400 μg/mL.

[00437] **Strains.** All experiments with *E. coli* in this Example were done with MG1655 (ATCC 700926) derived strains. The *recA*, *iscS* and TCA cycle knockouts were constructed using P1 phage transduction. Briefly, *recA::KanR* was transduced from an E. coli single-gene knockout library (T. Baba et al., Mol. Syst. Biol., 2006, 2:2006.0008) into the MG1655 strain. The kanamycin-resistance gene was removed using the pcp20 plasmid (K.A. Datsenko and B.L. Wanner, Proc. Natl. Acad. Sci. USA, 2000, 97: 664-6645) to create MG1655*ΔrecA*. This procedure was replicated with *iscS* and the TCA cycle knockouts to move these single gene knockouts into MG1655.

[00438] For work with *S. aureus*, a *Staphylococcus aureus* subspecies *aureus* Rosenback strain was used that was obtained from ATCC (29740).

[00439] **DNA damage sensor construction.** The DNA sensor construct with ampicillin resistance was described previously (D.J. Dwyer *et al.*, "Superoxide and hydroxyl radicals contribute to gyrase inhibitor-mediated cell death in E. coli", 2007 accepted by Mol. Syst. Biol., which is incorporated herein by reference in its entirety).

[00440] **Growth Conditions.** In the experiments, the growth and survival of untreated exponential phase *E. coli*, or *S. aureus*, were compared to cultured treated with antibiotics at the above concentrations. *E. coli* cultures were treated with norfloxacin, kanamycin, ampicillin, rifamycin SV, chloramphenical, tetracycline, erythromycin, or spectinomycin, where appropriate, *S. aureus* cultures were treated with norfloxacin, vancomycin or chloramphenical, where appropriate.

[00441] **Hydroxyl radical and DNA damage sensor experiments using the flow cytometer.** To monitor the occurrence of DNA damage, the engineered DNA damage sensor was used, which responds to auto-cleavage of LexA by activating expression of *gfpmut3b*. All the drug treatments and the no-drug controls were collected with both the

kanamycin- and ampicillin-resistant version of the plasmid, with the exception of the ampicillin treatment (kanamycin plasmid only) and the kanamycin treatment (ampicillin plasmid only). All data were collected using a Becton Dickinson FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) with a 488-nm argon laser and a 515- to 545-nm emission filter (FL1) at low flow rate. The following PMT voltage settings were used: E00 (FSC), 360 (SSC) and 700 (FL1). To detect hydroxyl radical formation, the fluorescent reporter dye, 3'-(p-hydroxyphenyl) fluorescein (HPF, Invitrogen, Carlsbad, CA) was used at a concentration of 5 μM (1:1000) dilution), which is oxidized by hydroxyl radicals with high specificity. The following PMT voltage settings were used: E00 (FSC), 360 (SSC) and 825 (FL1). Calibrite beads (Becton Dickinson) were used for instrument calibration.

[00442] Flow data were processed and analyzed with MATLAB (MathWorks, Natick, MA) to construct figures. At least 50,000 cells were collected for each sample. In all experiments, cells were grown as described above. Samples were taken immediately before addition of drug (time zero), then every hour for 3 hours. At each timepoint, approximately 106 cells were collected, washed once and resuspended in filtered 1x PBS, pH 7.2 (Fisher Scientific), and fluorescence was measured on the flow cytometer.

[00443] **NAD+/NADH extraction and NAD cycling assay.** Protocols for dinucleotide extraction and the cycling assay were based on protocols by Leonardo et al. (M.R. Leonardo et al., J. Bacteriol., 1996, 178: 6013-6018).

[00444] **Gene Expression Analysis.** The present Applicants compared the microarray-determined (Affymetrix E. coli Antisense2 genome arrays) mRNA profiles of *E. coli* MG1655 cultures in response to bactericidal (25 ng/mL norfloxacin, 5 μg/mL ampicillin and 5 μg/mL kanamycin), bacteriostatic (400 μg/mL spectonomycin), or no-drug treatment. For all experiments, cells were grown overnight, then diluted 1:1000 into 250 mL LB for collection of total RNA. At an OD600 of 0.3, the culture was split, 50 mL into 5, 250 mL flasks and antibiotics added as described above. Samples for microarray analysis were taken immediately before treatment (time zero), then at 30, 60 and 120 minutes post treatment. For bactericidal concentration of norfloxacin (250 ng/mL), a time-series profile was used that had previously been collected (D.J. Dwyer *et al.*, "Superoxide and hydroxyl radicals contribute to gyrase inhibitor-mediated cell death in E. coli", 2007 accepted by Mol. Syst. Biol., which is incorporated herein by reference in its entirety).

[00445] The resulting microarray*.CEL files were combined with *.CEL files from arrays which comprise the M3D compendium (http://m3d.bu.edu; J.J. Faith *et al.*, PloS Biol., 2007, 5,e8).and RMA-normalized (B.M. Bolstad et al., Bioinformatics, 2003, 19: 185-193) with RMAexpress, for a total of 524 RMA-normalized *E. coli* expression arrays. For each gene, that gene standard deviation of expression was calculated and used to construct the z-scale difference between that gene's normalized expression in a given experimental condition (bactericidal drug treatment) versus a control (bacteriostatic drug treatment).

[00446] This allowed to measure each gene's change in expression for a given experiment in units of standard deviation, a form of the z-test. For each time point each bactericidal experiment set (norfloxacin, ampicillin, kanamycin), Δz-scores were converted to p-values and significantly up- and down-regulated genes with a q-value (False Discovery Rate) < 0.05 were selected (J.D. Storey *et al.*, Proc. Natl. Acad. Sci. USA, 2003, 100: 9440-9445). The resultant gene lists was merged across all time points (set-union) to obtain a coarse profile of the difference in expression between a given bactericidal drug and spectinomycin. Finally, the Applicants determined the common set of all genes that were up- or down-regulated by bactericidal concentrations of norfloxacin, kanamycin, and ampicillin with respect to spectinomycin (3-way set-intersect). For an additional pathway-level insight, Gene Ontology-based enrichment of the up- and down-regulated gene list was performed using GO TermFinder, requiring pathway enrichment q-values to be <0.05 and setting the p-value estimation mode to boostrapping.

Results

[00447] Hydroxyl radical formation, via cycles of oxidation and reduction of quinolone class of antineoplastic drugs used in cancer therapeutics, has been shown to contribute to cell death in tumor cells. In the present study, the Applicants chose to investigate whether hydroxyl radical formation also contributes to antibiotic-induced cell death in bacteria. Using the dye hydroxyphenyl fluorescein, HPF, which is oxidized by hydroxyl radicals with high specificity, hydroxyl radical formation was first observed over a range of hydrogen peroxide concentrations known to induce hydroxyl radicals through the reduction of hydrogen peroxide by ferrous iron. In this manner, it was possible to establish the scale of hydroxyl radical formation detectable using this dye via flow cytometry.

[00448] Hydroxyl radical formation following exposure to the three major classes of bactericidal antibiotics in *E. coli* (Fig. 35a,b). Specifically, the present Applicants examined

killing by the quinolone (250 ng/mL, norfloxacin), β-lactam (5 μg/mL, ampicillin), and aminoglycoside (5 μg/mL kanamycin) classes. Each of the three different classes of bactericidal antibiotics was found to induce hydroxyl radical formation (Fig. 35b). In contrast, the five bacteriostatic drugs tested (Fig. 35c) including four different classes of ribosome inhibitors (chloramphenicol, spectinomycin, tetracycline, and the macrolide erythromycin), as well as an inhibitor of RNA polymerase (rifamycin SV, referred to as ridamycin), did not stimulate hydroxyl radical production (Fig. 35d).

[00449] The present Applications have then demonstrated that gram-positive, as well as gram-negative bacteria, produce hydroxyl radicals in response to bactericidal antibiotics. Hydroxyl radical formation was observed for a bacteriostatic drug (chloramphenical), a bactericidal drug (norfloxacin), and both lethal (5 μg/mL) and sub-lethal (1 μg/mL) concentrations of vancomycin (a gram-positive specific bactericidal drug), in a wild-type strain of *S. aureus*. As expected, an increase in hydroxyl radical production was observed for the norfloxacin treatment and for the lethal concentration of vancomycin, while hydroxyl formation was not observed for the chloramphenical treatment or the sub-lethal concentration of vancomycin..

[00450] To demonstrate that hydroxyl radical formation is an important component of norfloxacin-, ampicillin- and kanamycin-mediated killing, the present Applicants additionally treated drug-exposed wild-type *E. coli* with the iron chelator, 2,2'-dipyridyl. Application of iron chelators is an established means of blocking Fenton reaction-mediated hydroxyl radical formation by sequestering unbound iron. For the three classes of bactericidal drug treatments, a significant increase in survival was observed following addition of 2,2'-dipyridyl (fig. 36a, c, e), confirming that hydroxyl radicals are involved in bactericidal antibiotic-induced cell death. 2,2'-dipyridyl significantly reduced hydroxyl radical formation in norfloxacin-treated cultures, and there appears to be some recovery from the norfloxacin-induced growth arrest and DNA damage between 2 and 3 hours into the treatment in the presence of 2,2'-dipyridyl (Fig. 36a). Similarly, killing by ampicillin and kanamycin, respectively, is reduced to less than 0.5-logs following application of the iron chelator (Fig. 36c, e), and is accompanied by a significant reduction in hydroxyl radical formation. As expected, addition of the iron chelator to bacteriostatic drug-treated cultures, which do not stimulate hydroxyl radical production, had no effect on the growth-arresting properties of these bacteriostatic classes of drugs.

[00451] Addition of thiourea, a potent hydroxyl radical quencher, was then added to drug-treated cultures to directly block the harmful effects of hydroxyl radicals. The cultures treated with norfloxacin and thiourea were found to show a significant delay in cell death at 1 hour, and a near 1-log increase in survival at 3 hours relative to norfloxacin treatment alone (Fig. 36a). This increase in survival again correlates with a decrease in hydroxyl radical formation. Thiourea was able to reduce ampicillin-mediated killing (Fig. 36c) and hydroxyl radical formation to the same extent as 2,2'-dipyridyl. Thiourea was less efficient at mitigating bacterial cell death following kanamycin treatment (Fig. 36e), which is reflected by the capacity of thiourea to reduce, but not eliminate, kanamycin-mediated hydroxyl radical formation. Addition of the radical quencher to bacteriostatic drug-treated cultures had minimal effects on the growth-arresting properties of these bacteriostatic classes of drugs.

[00452] The results obtained with 2,2'-dipyridyl and thiourea indicate that hydroxyl radical formation and the Fenton reaction play a critical role in effective killing by quinolones, β-lactams and aminoglycosides. The ferrous iron required for hydroxyl radical formation could come from intracellular sources, such as iron storage proteins or iron-sulfur clusters. The Applicants examined the killing properties of the bactericidal drugs in a strain (*ΔiscS*) with significantly impaired iron-sulfur cluster synthesis capabilities and thus a large decrease in iron-sulfur cluster abundance, to determine if oxidative damage of iron-cluster is a key source of ferrous iron driving hydroxyl radical formation for bactericidal drugs. In this strain, a significant reduction in cell death and hydroxyl radical formation were observed following treatment with norfloxacin (Fig. 35a), ampicillin (Fig. 36c) or kanamycin (Fig. 36e). This strongly implies that intracellular ferrous iron is a key source for Fenton-mediated hydroxyl radical formation by bactericidal drugs.

[00453] It is interesting to consider how functionally distinct bactericidal drugs commonly stimulate damage to iron-sulfur clusters. The present Applications utilized gene expression microarray, and statistical analyses to find the common set of genes upregulated or downregulated by the bactericidal drugs norfloxacin, ampicillin and kanamycin, relative to the bacteriostatic drug spectinomycin. Pathway enrichment (q-value < 0.05) using gene ontology found NADH-coupled electron transport (NADH dehydrogenase I) as a key upregulated pathway common to all three bactericidal drug classes; a number of heat shock genes were also upregulated but did not appear in the pathway enrichment. The established mechanism underlying leaching of iron from iron-sulfur clusters predominantly occurs *via*

superoxide, and it is well accepted that the majority of superoxide generated in E. coli occurs through oxidation of the respiratory electron transport chain driven by oxygen and the conversion of reduced NADH and NAD^+.

[00454] Using a modified version (M.R. Leonardo et al., J. Bacteriol., 1996, 178: 6013-6018;) of the NAD^+ cycling assay developed by Bernofsky and Swan (C. Bernofsky *et al.*, Anal. Biochem., 1973, 53: 452-458), the present Applicants monitored NAD^+ and NADH concentrations in wildtype *E. coli* following treatment with the bactericidal drugs norfloxacin, ampicillin and kanamycin, as well as the bacteriostatic drug spectinomycin. For all three bactericidal drugs, a > 5 fold increase in the NAD^+/NADH ratio was observed 0.5 hours after drug addition. This ratio returned to untreated levels by 1 hour. The increase in the NAD^+/NADH ratio was predominantly due to a large relative drop in NADH accompanied by a modest surge in NAD^+. This spike was not observed in an untreated culture, where the ratio of NAD^+/NADH remained tightly bounded. More importantly, treatment with the bacteriostatic drug spectinomycin had no effect on the NAD^+/NADH ratio relative to the untreated culture. A surge in NADH consumption upon exposure to bactericidal antibiotics should induce a burst in superoxide generation via the respiratory chain. These events may account for destabilizing of iron-sulfur clusters, stimulation of the Fenton reaction and cell death.

[00455] NADH is generated from NAD^+ during the tricarboxylic acid (TCA) cycle. Therefore, loss of TCA cycle genes should reduce the available pool of NADH, decrease superoxide generation, and lead to increased survival following exposure to bactericidal drugs. Since NADH is produced at different points along the TCA cycle, the increase in survival should follow a gradient with loss of genes before production of the first reduced dinucleotide (*e.g.*, aconitase B (*acnB*) or icocitrate dehydrogenase (*icdA*)) leading to larger increases in survival than loss of genes after the various NADH producing steps in the TCA cycle (*e.g.*, 2-ketoglutarate dehydrogenase (*sucB*, *sucA*, *lpdA*), or malate dehydrogenase (*mdh*)). Blocking the TCA cycle before the formation of the first reduced dinucleotide (*ΔicdA* and *ΔacnB*), led to increased survival following norfloxacin treatment, which had the largest increase in NAD^+/NADH, whereas TCA cycle knockouts after this point (*ΔsucB* and *Δmdh*) behaved like wildtype. Blocking the TCA cycle through to the second NADH formation step (*ΔacnB, ΔicdA and ΔsucB*), led to increased survival following ampicillin treatment, while blocking the last NAHD formation step (*Δmdh*) did not affect survival.

Finally, each of the TCA cycle knockout strains (*ΔacnB, ΔicdA and ΔsucB and Δmdh*), exhibited increased survival following exposure to kanamycin.

[00456] It is important to note that aconitase A (AcnA) and aconitase B are the two main forms of aconitase in *E. coli*, and AcnB functions as the main catabolic enzyme in the TCA cycle while AcnA responds to oxidative stress. As expected, for all three classes of bactericidal drugs, increased survival was observed only with *ΔacnB*; *ΔacnA* behaved like wildtype. Interestingly, one of the first mutants selected for resistance to low levels of nalidixic acid, a quinolone, was mapped to a loss of isocitrate dehydrogenase (*icdA*) and later studies found the same following removal of both acnA and acnB. The surge in NADH consumption induced by bactericidal drugs, coupled with the phenotypic results from the TCA cycle knockouts, all point toward efficient metabolism as an important aspect of killing by bactericidal drugs. This also provides a mechanistic basis for the observation that carbon source limitation reduces the efficacy of killing by bactericidal drugs. Together, these results indicate that bactericidal drugs, for all of their diverse targets, stimulate hydroxyl radical formation through a common pathway.

[00457] Hydroxyl radical are extremely toxic, and will readily damage membrane lipids, proteins and DNA. Following application of bactericidal antibiotics, initiation of the DNA damage response system (SOS response) is expected to be observed, where RecA is activated by DNA damage, promoting auto-cleavage of the LexA repressor protein and stimulation of SOS-response genes. To examine SOS induction by the various bactericidal and bacteriostatic drugs used, an engineered promoter-reporter gene construct was used that expresses Green Fluorescent Protein (GFP) upon LexA auto-cleavage.

[00458] As expected, a significant increase in SOS activity was observed upon treatment with norfloxacin (Fig. 37a). β-lactams have recently been shown to induce expression of the SOS response mediator of filamentation, sulA, through the DpiBA two-component system. LexA-driven GFP expression showed that ampicillin induced the SOS response *via* RecA activation (Fig. 37a). Following kanamycin treatment, no change in SOS activity was observed over the course of the experiment (Fig. 37a). Since the reporter construct requires active transcription and translation to express GFP and LexA auto-cleavage, and kanamycin blocks translation, these data (Fig. 37a) do not rule out oxidative DNA damage following kanamycin treatment. Given the hydroxyl radical data obtained and the damage this oxidative molecule can cause to DNA, the present Applicants suspect that DNA damage is occurring and speculate that the binding of kanamycin to the ribosome additionally blocks translation of the SOS-regulated genes.

[00459] The present results concerning hydroxyl radical formation provide a mechanistic basis for the earlier findings that the killing effects of quinolones and β-lactams can be potentiated by knocking out *recA* and disabling the SOS response, which the present Applications confirmed as shown in Fig. 37b. To demonstrate that disabling the SOS response can also increase the potency of aminoglycosides, kanamycin's killing efficiency was examined in a recA knockout. In the *ΔrecA* strain compared to wildtype, a significantly increased rate of killing was observed at 1 and 2 hours following addition of kanamycin (Fig. 37b). These data highlight the importance of an intact DNA damage repair system for mitigating the effects of hydroxyl radical-mediated DNA damage induced by all three major classes of bactericidal antibiotics.

[00460] In the present study, the three major classes of bactericidal drugs were shown to utilize a common mechanism of killing whereby they stimulate the production of lethal doses of hydroxyl radicals. Through application of an iron chelator, quinolones, β-lactams and aminoglycosides were shown to stimulate hydroxyl radical formation *via* the Fenton reaction. Even more importantly, both the iron chelator and hydroxyl radical quencher attenuate killing by bactericidal drugs, indicating that hydroxyl radicals are a critical contributor to bactericidal antibiotic-mediated cell death. Using the *iscS* knockout, the present Applicants showed that iron-sulfur clusters are an important source of the iron required to stimulate Fenton-mediated hydroxyl radical formation.

[00461] Inhibition of DNA gyrase by quinolones, inhibition of penicillin-binding proteins by β-lactams, and inhibition of proper translation by aminoglycosides were shown to trigger a metabolic response that induces depletion of NADH. This, in turn, stimulates free radical damage of iron-sulfur clusters leading to exposure, or leaching of ferrous iron that stimulates the Fenton reaction, ultimately resulting in hydroxyl radical formation and cell death. Elucidation of the steps that occur after the diverse bactericidal drug-target interactions but before the step of NADH depletion will further our understanding of this common mechanism of killing.

[00462] Antibacterial drug design has focused on blocking essential cellular functions. This has yielded significant advances in antibacterial therapy; however, the ever-increasing prevalence of antibiotic-resistant strains has made it critical that we develop novel, more effective means of killing bacterial. The present results indicate that targeting bacterial systems that remediate hydroxyl radical damage, including proteins involved in triggering the DNA damage response, *e.g.*, RecA, is a viable means of potentiating all three major classes of bactericidal drugs. Moreover, pathway analyses and systems biology approaches may uncover drug gable targets for stimulating hydroxyl radical formation, which cold result in new classes of bactericidal antibiotics.

Other Embodiments

[00463] Other embodiments of the invention will be apparent to those skilled in the art from a consideration of the specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope of the invention being indicated by the following claims.

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Claims

What is claimed is:

1. A method comprising steps of:
    administering a RecA inhibitor to a subject suffering from or susceptible to a microbial infection; and
    administering an antibiotic agent to the subject.

2. The method of claim 1, wherein the RecA inhibitor is administered in an amount effective to potentiate activity of the antibiotic agent.

3. The method of claim 2, wherein the antibiotic agent is a bacteriocidal antibiotic agent.

4. The method of claim 2, wherein the antibiotic agent produces hydroxyl radicals.

5. The method of claim 1, wherien the RecA inhibitor is administered in an amount effective for suppression of resistance, such that resistance to the antibiotic agent occurs at a frequency below that observed under otherwise comparable conditions that lack RecA inhibitor administration.

6. The method of claim 1, wherein the RecA inhibitor is administered in an amount effective to retard resistance, such that resistance to the antibiotic agent occurs at a time later than that observed under otherwise comparable conditions that lack RecA inhibitor administration.

7. The method of claim 1, 5 or 6, wherein the subject is an animal.

8. The method of claim 1, 5 or 6, wherein the subject is a mammal.

9. The method of claim 1, 5 or 6, wherein the subject is a human.

10. The method of claim 1, 5 or 6, wherein the microbial infection is caused by bacteria, fungi or protists.

11. The method claim 1, 5 or 6, wherein the microbial infection is cause by gram positive or a gram negative bacteria.

12. The method of claim 1, 5 or 6, wherein the microbial infection is caused by acid fast bacteria.

13. The method of claim 1, 5 or 6, wherein the microbial infection is caused by bacteria that is sensitive to at least one antibiotic.

14. The method of claim 13, wherein the antibiotic agent administered to the subject and the antibiotic is sensitive belong to the same class of antibiotics.

15. The method of claim 14, wherein the antibiotic agent administered to the subject and the antibiotic are identical.

16. The method of claim 13, wherein the antibiotic agent administered to the subject and the antibiotic belong to different classes of antibiotics.

17. The method of claim 1, 5 or 6, wherein the microbial infection is caused by bacteria that show resistance to at least one antibiotic.

18. The method of claim 17, wherein the antibiotic agent administered to the subject and the antibiotic belong to the same class of antibiotics.

19. The method of claim 18, wherein the antibiotic agent administered to the subject and the antibiotic are identical.

20. The method of claim 17, wherein the antibiotic agent administered to the subject and the antibiotic belong to different classes of antibiotics.

21. The method of claim 1, 5 or 6, wherein the antibiotic agent administered to the subject is a member of the group consisting of an aminoglycoside, an aminomethylcycline, an aminophenicol, an ansamycin, a β-lactam, a carbapenem, a dapsone, a 2,4-diaminopyrimidine, a glycopeptide, a glycycycline, a ketolid, a lincomycin, a lincosamide, a macrolide, a nitrofuran, an oxazolidinone, a peptide, a polymyxin, a quinolone, a rifabutin, a streptogramin, a sulfonamide, a sulfones a tetracycline, and combinations thereof.

22. The method of claim 1, 5 or 6, wherein the antibiotic agent administered to the subject comprises a quinolone.

23. The method of claim 22, wherein the antibiotic agent administered to the subject comprises a fluoroquinolone.

24. The method of claim 1, 5 or 6, wherein the antibiotic agent administered to the subject comprises an aminoglycoside.

25. The method of claim 1, 5 or 6, wherein the antibiotic agent administered to the subject comprises a cyclic peptide antibiotic.

26. The method of claim 25, whereint the antibiotic agent administered to the subject comprises a polymyxin.

27. The method of claim 1, 5 or 6, wherein the antibiotic agent administered to the subject comprises a β-lactam.

28. The method of claim 1, 5 or 6, wherein the RecA inhibitor interacts directly with the RecA protein.

29. The method of claim 28, wherein the RecA inhibitor binds to at least one binding site on the RecA protein.

30. The method of claim 1, 5 or 6, wherein the RecA inhibitor competes with hinokiflavone for binding to RecA.

31. The method of claim 1, 5 or 6, wherein the RecA inhibitor binds to more than one binding site on the RecA protein.

32. The method of claim 1, 5 or 6, wherein the RecA inhibitor inhibits at least one activity of RecA, wherein the RecA activity is a member of the group consisting of DNA binding, monomer interaction, helicase activity, filament formation, ATP binding, ATP hydrolysis, co-protease activity, recombinase activity, and replication function.

33. The method of claim 1, 5 or 6, wherein the RecA inhibitor inhibits ATPase activity.

34. The method of claim 1, 5 or 6, wherein the RecA inhibitor has a IC_{50} of less than about 100 μg/mL, less than about 50 μg/mL, less than about 15 μg/mL, less than

about 10 μg/mL, less than about 5 μg/mL, less than about 3 μg/mL, or less than about 1 μg/mL.

35. The method of claim 1, 5 or 6, wherein the RecA inhibitor and antibiotic agent are administered substantially simultaneously.

36. The method of claim 1, 5 or 6, wherein the RecA inhibitor and antibiotic agent are administered sequentially.

37. The method of claim 1, 5 or 6, wherein the RecA inhibitor is administered prior to administration of the antibiotic agent.

38. The method of claim 1, 5 or 6, wherein the RecA inhibitor and antibiotic agent are administered using identical administration routes.

39. The method of claim 1, 5 or 6, wherein the RecA inhibitor and antibiotic agent are administered using different administration routes.

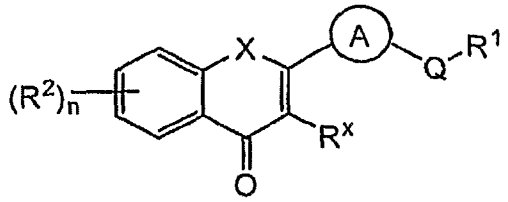
40. The method of claim 1, 5 or 6, wherein the antibiotic agent is administered at a dose below its conventional dose.

41. The method of claim 1, 5 or 6, wherein the antibiotic agent is administered at a frequency below that of its conventional administration.

42. The method of claim 1, 5 or 6, wherein the antibiotic agent is administered at a dose below its conventional dose and at a frequency below that of its conventional administration.

43. The method of claim 1, 5 or 6, wherein administration of the RecA inhibitor and antibiotic agent results in less toxicity that administration of the antibiotic agent alone.

44. The method of claim 1, 5 or 6, wherein the RecA inhibitor has the following structure:



I

or a pharmaceutically acceptable salt or derivative thereof, wherein:

X is oxygen, sulfur, or N(R);

n is 0 to 4;

R^1 is hydrogen, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

each R^2 is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$;

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

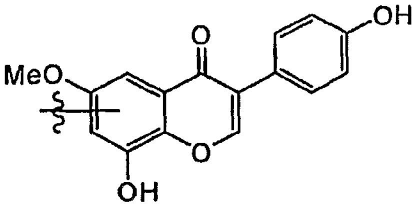
each R is independently hydrogen or an optionally substituted aliphatic group;

R^x is R or OR; and

Ring A is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8–10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

45. The method of claim 44, wherein X is oxygen.

46. The method of claim 44, wherein R^1 has the following structure:



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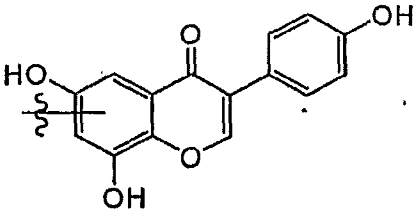

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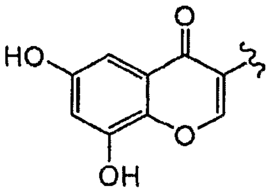

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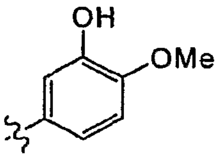
wherein each wavy line depicts the point of attachment to Q.

47. The method of claim 44, wherein R^1 has the following structure:



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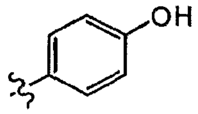

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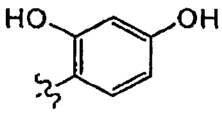

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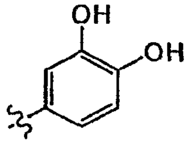

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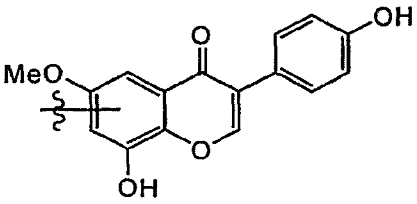
wherein each wavy line indicates the point of attachment to Q.

48. The method of claim 44, wherein R^2 is selected from the group consisting of: OH, OMe,



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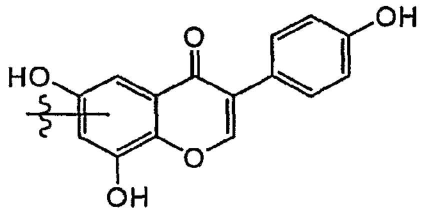

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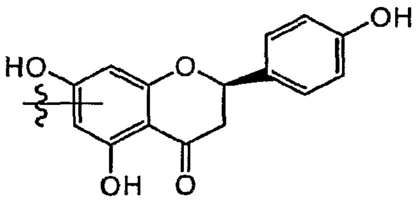

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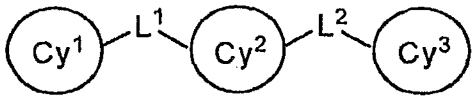
wherein each wavy line depicts the point of attachment.

49. The method of claim 1, 5 or 6, wherein the RecA inhibitor has the following structure:



II

or a pharmaceutically acceptable salt or derivative thereof, wherein:

Cy^1 is a an optionally substituted 5-6 membered aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^1 is a valence bond, a C_{1-6} bivalent saturated, unsaturated, straight or branched hydrocarbon chain, -N(R)-, -N(R)SO_2-, -N(R)SO_2N(R)-, -N(R)C(O)-, -C(O)N(R)-, or -N(R)C(O)N(R)-;

each R is independently hydrogen or an optionally substituted C_{1-6} aliphatic group;

Cy^2 is an optionally substituted 6-membered aryl ring having 0-2 nitrogen atoms, an 8-10 membered bicyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 5-membered heteroaryl ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^2 is a C_{1-6} bivalent saturated, unsaturated, straight or branched hydrocarbon chain, -CH_2CH_2C(=W)N(R)N(R)C(=W)-, -N(R)C(=W)N(R)C(=W)C$(R)_2$W-, -C(=W)N(R)N(R)C(=W)N(R)-, -C(=W)N(R)N(R)C(=W)N(R)CH=CH_2, or -C(=W)N(R)C(=W)N(R)-, wherein each W is independently oxygen or sulfur; and

Cy^3 is an optionally substituted 6-membered aryl ring having 0-2 nitrogen atoms.

50. The method of claim 1, 5 or 6, wherein the RecA inhibitor has one of the structures presented in Figure 14, Figure 15 and Figure 16.

51. The method of claim 1, 5 or 6, wherein the RecA inhibitor is selected from the group consisting of amentoflavone, apigenin, apigenin-7-O-glucoside, fitsetin, hinokiflavone, 6-hydroxyapigenin, isorhamnetin, kaempferol, maclurin, morin, quercetagetin, quercetin dihydrate, 3,7,4'-trihydroxyflavone, fisetin, theaflavin, and combinations thereof.

52. The method of claim 1, 5 or 6 futher comprising a step of administering at least one additional antibitiotic agent to the subject.

53. A pharmaceutical composition comprising a RecA inhibitor, an antibiotic agent, and at least one physiologically acceptable carrier or excipient, wherein, when administered to a subject suffering from or susceptible to a microbial infection, the RecA inhibitor potentiates activity of the antibiotic agent.

54. The pharmaceutical composition of claim 53, wherein the antibiotic agent is a bactericidal antibiotic agent.

55. The pharmaceutical composition of claim 53, wherein the antibiotic agent produces hydroxyl radicals.

56. A pharmaceutical composition comprising a RecA inhibitor, an antibiotic agent, and at least one physiogically acceptable carrier or excipient, wherein, when administered to a subject suffering from or susceptible to a microbial infection, the RecA inhibitor suppresses resistance, such that resistance to the antibiotic agent occurs at a frequency below that observed under otherwise comparable conditions that lack RecA inhibitor administration.

57. A pharmaceutical composition comprising a RecA inhibitor, an antiobiotic agent, and at least one physiologically acceptable carrier or excipient, wherein when administered to subject suffering from or susceptible to a microbial infection, the RecA inhibitor retards resistance, such that resistance to the antibiotic agent occurs at a time later than that observed under otherwise comparable conditions that lack RecA inhibitor administration.

58. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor and antibiotic agent are formulated for substantially simultaneous administration or for sequential administration.

59. The pharmaceutical composition of claim 53, 56 or 57, wherein the antibiotic agent is a member of the group consisting of an aminoglycoside, an aminomethylcycline, an aminophenicol, an ansamycin, a β-lactam, a carbapenem, a dapsone, a 2,4-diaminopyrimidine, a glycopeptide, a glycycycline, a ketolid, a lincomycin, a lincosamide, a macrolide, a nitrofuran, an oxazolidinone, a peptide, a polymyxin, a quinolone, a rifabutin, a streptogramin, a sulfonamide, a sulfones a tetracycline, and combinations thereof.

60. The pharmaceutical composition of claim 53, 56 or 57, wherein the antibiotic agent comprises a quinolone.

61. The pharmaceutical composition of claim 53, 56 or 57, wherein the antibiotic agent comprises a fluoroquinolone.

62. The pharmaceutical composition of claim 53, 56 or 57, wherein the antibiotic agent comprises an aminoglycoside.

63. The pharmaceutical composition of claim 53, 56 or 57, wherein the antibiotic agent comprises a cyclic peptide antibiotic.

64. The pharmaceutical composition of claim 53, 56 or 57, wherein the antibiotic agent comprises a polymyxin.

65. The pharmaceutical composition of claim 53, 56 or 57, wherein the antibiotic agent comprises a β-lactam.

66. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor interacts directly with the RecA protein.

67. The pharmaceutical composition of claim 66, wherein the RecA inhibitor binds to at least one binding site on the RecA protein.

68. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor competes with hinokiflavone for binding to RecA.

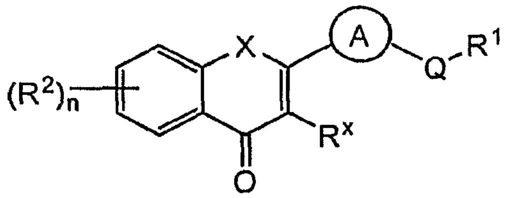
69. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor binds to more than one binding site on the RecA protein.

70. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor inhibits at least one activity of RecA, wherein the RecA activity is a member of the group consisting of DNA binding, monomer interaction, helicase activity, filament formation, ATP binding, ATP hydrolysis, co-protease activity, recombinase activity, and replication function.

71. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor inhibits ATPase activity.

72. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor has a IC_{50} of less than about 100 μg/mL, less than about 50 μg/mL, less than about 15 μg/mL, less than about 10 μg/mL, less than about 5 μg/mL, less than about 3 μg/mL, or less than about 1 μg/mL.

73. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor has the following structure:



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or a pharmaceutically acceptable salt or derivative thereof, wherein:

X is oxygen, sulfur, or N(R);

n is 0 to 4;

R^1 is hydrogen, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

each R^2 is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$;

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

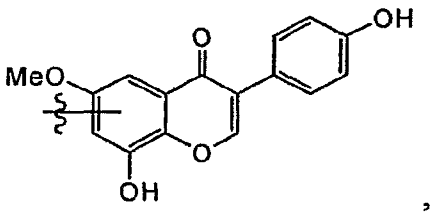
each R is independently hydrogen or an optionally substituted aliphatic group;

R^x is R or OR; and

Ring A is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8–10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

74. The pharmaceutical composition of claim 73, wherein X is oxygen.

75. The pharmaceutical composition of claim 73, wherein R^1 has the following structure:



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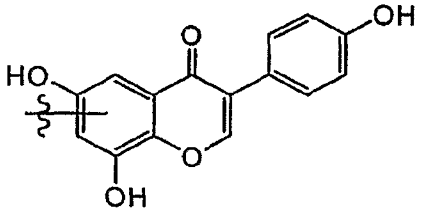
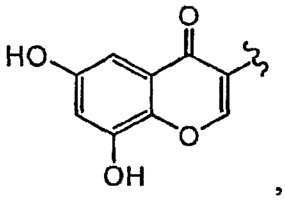


, or



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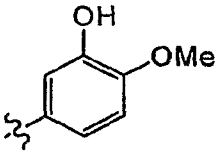
wherein each wavy line depicts the point of attachment to Q.

76. The pharmaceutical composition of claim 73, wherein R^1 has the following structure:



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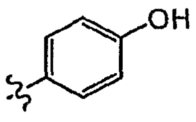

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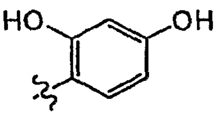
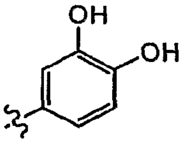


, or



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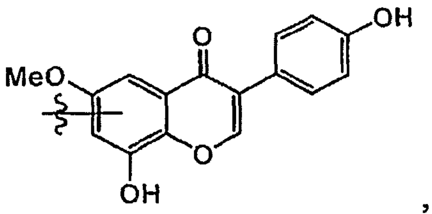
wherein each wavy line indicates the point of attachment to Q.

77. The pharmaceutical composition of claim 73, wherein R^2 is selected from the group consisting of: OH, OMe,



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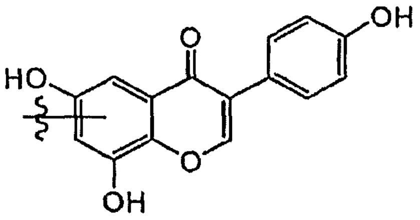
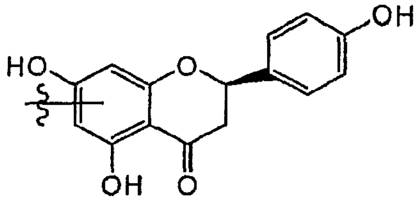


, and



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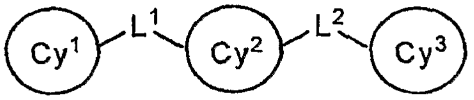
wherein each wavy line depicts the point of attachment.

78. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor has the following structure:



II

or a pharmaceutically acceptable salt or derivative thereof, wherein:

Cy^1 is a an optionally substituted 5-6 membered aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^1 is a valence bond, a C_{1-6} bivalent saturated, unsaturated, straight or branched hydrocarbon chain, -N(R)-, -N(R)SO_2-, -N(R)SO_2N(R)-, -N(R)C(O)-, -C(O)N(R)-, or -N(R)C(O)N(R)-;

each R is independently hydrogen or an optionally substituted C_{1-6} aliphatic group;

Cy^2 is an optionally substituted 6-membered aryl ring having 0-2 nitrogen atoms, an 8-10 membered bicyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 5-membered heteroaryl ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

L^2 is a C_{1-6} bivalent saturated, unsaturated, straight or branched hydrocarbon chain, -CH_2CH_2C(=W)N(R)N(R)C(=W)-, -N(R)C(=W)N(R)C(=W)C$(R)_2$W-, -C(=W)N(R)N(R)C(=W)N(R)-, -C(=W)N(R)N(R)C(=W)N(R)CH=CH_2, or -C(=W)N(R)C(=W)N(R)-, wherein each W is independently oxygen or sulfur; and

Cy^3 is an optionally substituted 6-membered aryl ring having 0-2 nitrogen atoms.

79. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor has one of the structures presented in Figure 14, Figure 15 and Figure 16.

80. The pharmaceutical composition of claim 53, 56 or 57, wherein the RecA inhibitor is selected from the group consisting of amentoflavone, apigenin, apigenin-7-O-glucoside, fitsetin, hinokiflavone, 6-hydroxyapigenin, isorhamnetin, kaempferol, maclurin, morin, quercetagetin, quercetin dihydrate, 3,7,4'-trihydroxyflavone, fisetin, theaflavin, and combinations thereof.

81. The pharmaceutical composition of claim 53, 56 or 57 futher comprising at least one additional antibitiotic agent.

82. A composition comprising an antibiotic agent and an agent that inhibits activity of an expression product of an antibiotic potentiator target gene.

83. The composition of claim 82, wherein the antibiotic potentiator target gene is a nonessential gene in cell culture.

84. The composition of claim 82, wherein the antibiotic potentiator target gene impairs both growth and survival of microbial cells under standard culture conditions in the presence of a sublethal concentration of the antibiotic agent but does not substantially impair growth or survival of microbial cells under standard culture conditions in the absence of the antibiotic agent.

85. The composition of claim 82, wherein the antibiotic agent is an inhibitor of a microbial type II topoisomerase and the antibiotic potentiator target gene is a quinolone potentiator target gene.

86. The composition of claim 85, wherein the quinolone potentiator target gene encodes a microbial DNA metabolizing enzyme or a subunit thereof.

87. The composition of claim 85, wherein the quinolone potentiator target gene encodes a microbial helicase or a subunit thereof.

88. The composition of claim 85, wherein the quinolone potentiator target gene is selected from the group consisting of *recA, recB, recC, recG, recN, ruvA, ruvB, ruvC,*

uvrD, xerD, xseA, xseB, pinQ, dnaT, priA, polA, thyA, b1440, fabH, dksA, fis, dapF, fliN, jw5303, yhfT, yqgC, ycjS, resA, dapF, ygoC, rimK, and *yicN.*

89. The composition of claim 85, wherein the quinolone potentiator target gene is selected from the group consisting of *dapF, fabH, fis, fliN, jw5303, priA, recA, recB, recC, recG, resA, ruvA, ruvB, ruvC, ruvC, uvrD, ycjS,ydfL, yhfT,* and *yqgC.*

90. The composition of claim 85, wherein the agent that inhibits activity of an expression product of a quinolone potentiator target gene is an ATPase inhibitor.

91. The composition of claim 85, wherein the agent that inhibits activity of an expression product of a quinolone potentiator target gene is a helicase inhibitor.

92. The composition of claim 85, whereint he the agent that inhibits activity of an expression product of a quinolone potentiator target gene inhibits RecA.

93. The composition of claim 85, whereint he the agent that inhibits activity of an expression product of a quinolone potentiator target gene inhibits ResA.

94. The composition of claim 85, wherein the inhibitor of a microbial type II topoisomerase is a quinolone antibiotic.

95. The composition of claim 85, wherein the inhibitor of a microbial type II topoisomerase is a fluoroquinolone antibiotic.

96. The composition of claim 82, wherein the antibiotic agent is an aminoglycoside and the antibiotic potentiator target gene is an aminoglycoside potentiator target gene.

97. The composition of claim 96, wherein the aminoglycoside potentiator target gene is selected from the group consisting of *aceE, araJ, aspA, atpA, atpF, atpH, brnQ, cydB, degP, dnaT, fepD, folP, guaB, Hfq, iscS, JW5360, lipA, lpdA, nuoB, nuoJ, nuoL, osmB, pdxH, pnuC, priA, priB, rbfA, recA, rfaD, rpmJ, rpoD, resA, ruvC, sdhC, seqA, setA, sucB, surA, tar, tatE, tauB, trmU, ubiH, ubiX, xerC, yaaU, ycfM, yejO, ygfZ, ygiH, yheL, yheM, yheN, yicG, yjdL, yjeT, yjiO,* and *yjjY.*

98. The composition of claim 96, wherein the aminoglycoside potentiator target gene is selected from the group consisting of *priA, recA, resA, sucB, ruvC, ubiH and ubiX,*

ycfM, ygfZ, yheL and yheM, atpA, atpF, and atpH, iscS, JW5360, guaB, lipA, pdxH, resA, pnuC, secY, secE, and *secG.*

99. The composition of claim 96, wherein the agent that inhibits activity of an expression product of an aminoglycoside potentiator target gene is an ATPase inhibitor.

100. The composition of claim 96, wherein the agent that inhibits activity of an expression product of an aminoglycoside potentiator target gene inhibits RecA.

101. The composition of claim 82, wherein the antibiotic agent is a peptide antibiotic and the antibiotic potentiator target gene is a peptide antibiotic potentiator target gene.

102. The composition of claim 101, wherein the peptide antibiotic potentiator target gene is selected from the group consisting of *agaAk, atpA, atpF, atpH, bglF, cysE, cysI, fepC, fepD, frvR, guaA, guaB, hofF, hsdS, iscS, JW4016, JW5075, JW5227, JW5257, JW5360, kdgK, lipA, lysA, malG, mbhA, mdoG, Nei, nmpC, nudH, pdxH, phnB, phnL, phnO, pnuC, potE, pshM, ptsA, rhaT, rpiA, resA, Sbp, speA, sucB, sugE, tdcE, tdcG, tolC, trxA, ubiE, ubiH, ubiX, Xni, ybbY, ycfM, ydeJ, yeeY, yfeT, ygaA, ygfZ, yhdX, yheL, yheM, yiaY, yidK, yihV, yjbN, yjcR, yjcZ, ynjD, yqeC, yqiH,* and *yrfA.*

103. The composition of claim 101, wherein the peptide antibiotic potentiator target gene is selected from the group consisting of *sucB, ubiH and ubiX, ycfM, ygfZ, yheL and yheM, atpA, atpF, and atpH, iscS, JW5360, guaB, lipA, pdxH, resA* and *pnuC.*

104. The composition of claim 101, wherein the agent that inhibits activity of an expression product of a peptide antibiotic potentiator target gene is an ATPase inhibitor.

105. The composition of claim 101, wherein the agent that inhibits activity of an expression product of a peptide antibiotic potentiator target gene inhibits RecA.

106. The composition of claim 82, wherein the therapeutic index of the composition is greater than the therapeutic index of the antibiotic agent.

107. A method of inhibiting growth or survival of a microbial cell comprising steps of:

contacting the microbial cell with an antibiotic agent; and
contacting the cell with an agent that potentiates activity of the antibiotic agent.

108. The method of claim 107, wherein the agent that potentiates activity of the antibiotic agent inhibits or interferes with activity of an expression product of an antibiotic potentiator target gene.

109. The method of claim 107, wherein the contacting occurs in cell culture.

110. The method of claim 107, wherein the contacting occurs in or on a multicellular organism.

111. The method of claim 107, wherein the cell is contacted with the antibiotic agent at a concentration lower than the MIC of the antibiotic agent against the microbial cell, and wherein the growth and survival of the microbial cell is inhibited at least as effectively as would be the case if the microbial cell was contacted with the antibiotic agent at a concentration greater than the MIC of the antibiotic agent against the microbial cell in the absence of the agent that potentiates activity of the antibiotic agent.

112. The method of claim 107, wherein the agent that potentiates activity of the antibiotic agent does not significantly impair growth of the microbial cell at a concentration at which it potentiates activity of the antiobiotic agent by a factor of 2.

113. The method of claim 107, wherein the antibiotic agent is a quinolone, an aminoglycoside, a peptide antibiotic, or a β-lactam.

114. A method of identifying an antibiotic potentiator target gene comprising steps of:
contacting a microbial cell with an antibiotic agent, wherein the microbial cell has a genetic alteration that significantly reduces or eliminates expression of a gene;
comparing growth or survival of the microbial cell with growth or survival of a microbial cell having higher expression of the gene than the cell having the genetic alteration; and
determining that the gene is an antibiotic potentiator target gene if the growth, survival, or both of the microbial cell having the genetic alteration is lower than

the growth, survival, or both of the microbial cell having higher expression of the gene.

115. The method of claim 114, wherein the microbial cell is contacted with the antibiotic agent at a concentration below the MIC of the antibiotic agent for the microbial cell.

116. The method of claim 114, wherein the microbial cell is contacted with the antibiotic agent at a non-inhibitory concentration.

117. The method of claim 114, wherein the method is performed on a collection of strains of a microbial species, each of which has a genetic alteration that significantly reduces or eliminates expression of a different gene.

118. The method of claim 114 further comprising steps of:

contacting an assay system with a test compound, wherein the assay system is suitable for performing an assay to detect expression of the antibiotic potentiator target gene or to detect activity of an expression product of the antibiotic potentiator target gene; and

determining whether the test compound inhibits expression of the antibiotic potentiator target gene or inhibits or interferes with activity of an expression product of antibiotic potentiator target gene.

119. An assay system comprising:

at least one test compound; and

components suitable for performing an assay that detects expression of an antibiotic potentiator target gene or detects activity of an expression product of an antibiotic potentiator target gene.

120. The assay system of claim 119, wherein the antibiotic potentiator gene is selected from the group consisting of *dapF, fabH, fis, fliN, jw5303, priA, recA, recB, recC, recG, resA, ruvA, ruvB, ruvC, uvrD, ycjS, ydfL, yhfT, yqgC, aceE, araJ, aspA, atpA, atpF, atpH, brnQ, cydB, degP, dnaT, fepD, folP, guaB, Hfq, iscS, JW5360, lipA, lpdA, nuoB, nuoJ, nuoL, osmB, pdxH, pnuC, priB, rbfA, rfaD, rpmJ, rpoD, sdhC, seqA, setA, sucB, surA, tar, tatE, tauB, trmU, ubiH, ubiX, xerC, yaaU, ycfM, yejO, ygfZ, ygiH, yheL, yheM, yheN, yicG, yjdL, yjeT, yjiO, yjjY, agaAk, bglF, cysE, cysI, fepC, frvR, guaA, hofF, hsdS, JW4016, JW5075, JW5227, JW5257, kdgK, lysA,*

malG, mbhA, mdoG, Nei, nmpC, nudH, phnB, phnL, phnO, potE, pshM, ptsA, rhaT, rpiA, Sbp, speA, sugE, tdcE, tdcG, tolC, trxA, ubiE, Xni, ybbY, ydeJ, yeeY, yfeT, ygaA, yhdX, yiaY, yidK, yihV, yjbN, yjcR, yjcZ, ynjD, yqeC, yqiH, and *yrfA.*

121. The assay system of claim 119, wherein the antibiotic potentiator gene is selected from the group consisting of *priA, recA, ruvC, sucB, ubiH and ubiX, ycfM, ygfZ, yheL and yheM, atpA, atpF, and atpH, iscS, JW5360, guaB, lipA, pdxH, resA* and *pnuC.*

122. The assay system of claim 119, wherein the assay is a cell-free assay.

123. The assay system of claim 119, wherein the assay is an ATPase assay.

124. The assay system of claim 119, wherein the assay measures a step in a bacterial fatty acid biosynthesis pathway.

125. The assay system of claim 119 further comprising an isolated polypeptide encoded by the antibiotic potentiator target gene.

126. The assay system of claim 119 further comprising an isolated polypeptide encoded by the antibiotic potentiator target gene, ATP, and means for detecting ATP or ATP hydrolysis.

127. The assay system of claim 119 further comprising an isolated recA polypeptide, ATP, and means for detecting ATP or ATP hydrolysis.

128. The assay system of claim 119 further comprising an isolated recA polypeptide, ATP, and luciferin.

129. The assay system of claim 119 further comprising a reporter construct.

130. The assay system of claim 119, wherein the assay is a fluorescence-based assay.

131. The assay system of claim 119, wherein the assay is a cell-based assay.

132. A method of identifying an agent that potentiates activity of an antibiotic comprising steps of:

providing an assay system comprising a test compound, wherein the assay system is suitable for performing an assay to detect expression of an antibiotic potentiator target gene or to detect activity of an expression product of an antibiotic potentiator target gene;

determining whether the test compound inhibits expression of the antibiotic potentiator target gene or inhibits or interferes with activity of an expression product of the antibiotic potentiator target gene; and

identifying the test compound as a potentiator of the antibiotic if the test compound inhibits expression of the antibiotic potentiator target gene or inhibits or interferes with activity of an expression product of the antibiotic potentiator target gene.

133. The method of claim 132, wherein the determining step comprising a step of:

comparing activity of the antibiotic potentiator target gene in the presence of the test compound with activity of the antibiotic potentiator target gene in the absence of the test compound.

134. The method of claim 132, wherein the test compound is present in the assay system at a first concentration and the determining step comprising a step of:

comparing activity of the antibiotic potentiator target gene in the presence of the first concentratino of the test compound with activity of the antibiotic potentiator target gene in the presence of a second concentration of the test compound, wherein the second concentration is lower than the first concentration.

135. The method of claim 132, wherein the identifying step comprises a step of:

identifying the test compound as a potentiator of the antibiotic if activity of the expression product of the antibiotic potentiator target gene is lower in the presence of the test compound than in its absence.

136. The method of claim 132, wherein the antibiotic potentiator gene is selected from the group consisting of *dapF, fabH, fis, fliN, jw5303, priA, recA, recB, recC, recG, resA, ruvA, ruvB, ruvC, uvrD, ycjS, ydfL, yhfT, yqgC, aceE, araJ, aspA, atpA, atpF, atpH, brnQ, cydB, degP, dnaT, fepD, folP, guaB, Hfq, iscS, JW5360, lipA, lpdA, nuoB, nuoJ, nuoL, osmB, pdxH, pnuC, priB, rbfA, rfaD, rpmJ, rpoD, sdhC, seqA,*

setA, sucB, surA, tar, tatE, tauB, trmU, ubiH, ubiX, xerC, yaaU, ycfM, yejO, ygfZ, ygiH, yheL, yheM, yheN, yicG, yjdL, yjeT, yjiO, yjjY, agaAk, bglF, cysE, cysI, fepC, frvR, guaA, hofF, hsdS, JW4016, JW5075, JW5227, JW5257, kdgK, lysA, malG, mbhA, mdoG, Nei, nmpC, nudH, phnB, phnL, phnO, potE, pshM, ptsA, rhaT, rpiA, Sbp, speA, sugE, tdcE, tdcG, tolC, trxA, ubiE, Xni, ybbY, ydeJ, yeeY, yfeT, ygaA, yhdX, yiaY, yidK, yihV, yjbN, yjcR, yjcZ, ynjD, yqeC, yqiH, and *yrfA.*

137. The method of claim 132, wherein the antibiotic potentiator gene is selected from the group consisting of *priA, recA, ruvC, sucB, ubiH and ubiX, ycfM, ygfZ, yheL and yheM, atpA, atpF, and atpH, iscS, JW5360, guaB, lipA, pdxH, resA* and *pnuC.*

138. The method of claim 132, wherein the assay is a cell-free assay.

139. The method of claim 132, wherein the assay is an ATPase assay.

140. The method of claim 132, wherein the assay utilizes a reporter construct.

141. The method of claim 132, wherein the assay a fluorescence-based assay.

142. The method of claim 132, wherein the assay comprises using an isolated polypeptide encoded by the antibiotic potentiator target gene.

143. The method of claim 132, wherein the assay is a cell-based assay.

144. A method of identifying a candidate antibiotic potentiating agent comprising steps of

contacting a protein comprising a polypeptide encoded by an antibiotic potentiator target gene with a test compound;
determining whether the test compound binds to the protein; and
identifying the test compound as an antibiotic potentiating agent if it binds to the protein.

145. The method of claim 144, wherein binding of the test compound to the protein is detected byu a method selected from the group consisting of: a competition binding assay, an immunoassay, and a three-hybrid assay.

146. The method of claim 144 further comprising steps of:

contacting the test compound that binds to the protein with a microbial cell and an antibiotic agent; and

determining whether the test compound potentiates the antibiotic agent.

147. The assay system of claim 144, wherein the antibiotic potentiator gene is selected from the group consisting of *dapF, fabH, fis, fliN, jw5303, priA, recA, recB, recC, recG, resA, ruvA, ruvB, ruvC, uvrD, ycjS, ydfL, yhfT, yqgC, aceE, araJ, aspA, atpA, atpF, atpH, brnQ, cydB, degP, dnaT, fepD, folP, guaB, Hfq, iscS, JW5360, lipA, lpdA, nuoB, nuoJ, nuoL, osmB, pdxH, pnuC, priB, rbfA, rfaD, rpmJ, rpoD, sdhC, seqA, setA, sucB, surA, tar, tatE, tauB, trmU, ubiH, ubiX, xerC, yaaU, ycfM, yejO, ygfZ, ygiH, yheL, yheM, yheN, yicG, yjdL, yjeT, yjiO, yjjY, agaAk, bglF, cysE, cysI, fepC, frvR, guaA, hofF, hsdS, JW4016, JW5075, JW5227, JW5257, kdgK, lysA, malG, mbhA, mdoG, Nei, nmpC, nudH, phnB, phnL, phnO, potE, pshM, ptsA, rhaT, rpiA, Sbp, speA, sugE, tdcE, tdcG, tolC, trxA, ubiE, Xni, ybbY, ydeJ, yeeY, yfeT, ygaA, yhdX, yiaY, yidK, yihV, yjbN, yjcR, yjcZ, ynjD, yqeC, yqiH,* and *yrfA.*

148. The assay system of claim 144, wherein the antibiotic potentiator gene is selected from the group consisting of *priA, recA, ruvC, sucB, ubiH and ubiX, ycfM, ygfZ, yheL and yheM, atpA, atpF, and atpH, iscS, JW5360, guaB, lipA, pdxH, resA* and *pnuC.*

149. A method of identifying a candidate antibiotic potentiating agent comprising steps of

contacting a first microbial cell with a test compound and an antibiotic agent, wherein the microbial cell overexpresses an antibiotic potentiator target gene;

comparing growth or survival of the first microbial cell with growth or survival, respectively, of a second microbial cell maintained under substantially equivalent conditions, wherein the second microbial cell does not overexpress the antibiotic potentiator target gene but is otherwise substantially identical to the first microbial cell ; and

determining that the test compound potentiates activity of the antibiotic agent if the growth, survival, or both of the first microbial cell is greater than the growth, survival, or both, of the second microbial cell

150. The assay system of claim 149, wherein the antibiotic potentiator gene is selected from the group consisting of *dapF, fabH, fis, fliN, jw5303, priA, recA, recB, recC, recG, resA, ruvA, ruvB, ruvC, uvrD, ycjS, ydfL, yhfT, yqgC, aceE, araJ, aspA, atpA, atpF, atpH, brnQ, cydB, degP, dnaT, fepD, folP, guaB, Hfq, iscS, JW5360, lipA, lpdA, nuoB, nuoJ, nuoL, osmB, pdxH, pnuC, priB, rbfA, rfaD, rpmJ, rpoD, sdhC, seqA, setA, sucB, surA, tar, tatE, tauB, trmU, ubiH, ubiX, xerC, yaaU, ycfM, yejO, ygfZ, ygiH, yheL, yheM, yheN, yicG, yjdL, yjeT, yjiO, yjjY, agaAk, bglF, cysE, cysI, fepC, frvR, guaA, hofF, hsdS, JW4016, JW5075, JW5227, JW5257, kdgK, lysA, malG, mbhA, mdoG, Nei, nmpC, nudH, phnB, phnL, phnO, potE, pshM, ptsA, rhaT, rpiA, Sbp, speA, sugE, tdcE, tdcG, tolC, trxA, ubiE, Xni, ybbY, ydeJ, yeeY, yfeT, ygaA, yhdX, yiaY, yidK, yihV, yjbN, yjcR, yjcZ, ynjD, yqeC, yqiH,* and *yrfA.*

151. The assay system of claim 149, wherein the antibiotic potentiator gene is selected from the group consisting of *priA, recA, ruvC, sucB, ubiH and ubiX, ycfM, ygfZ, yheL and yheM, atpA, atpF, and atpH, iscS, JW5360, guaB, lipA, pdxH, resA and pnuC.*

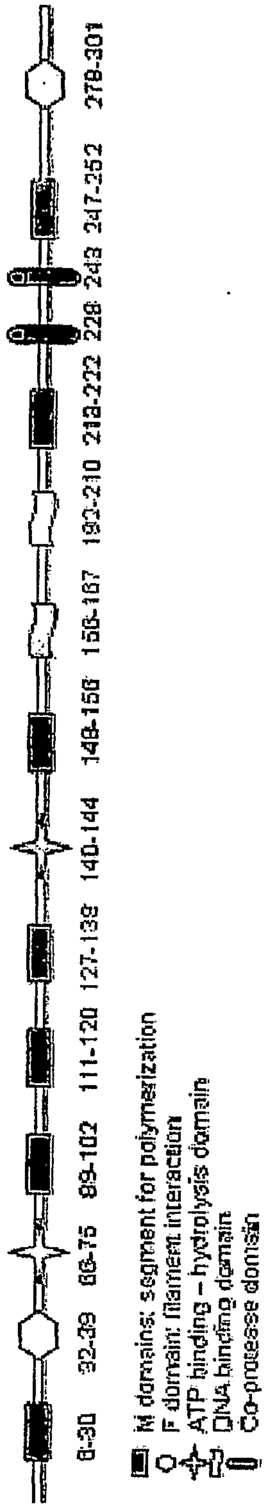

6-30
32-39
66-75
89-102
111-120
127-139
140-144
148-156
158-187
192-210
218-222
228
243
247-252
278-301
M domains: segment for polymerization
F domain: filament interaction
ATP binding – hydrolysis domain
DNA binding domain
Co-protease domain

Figure 1

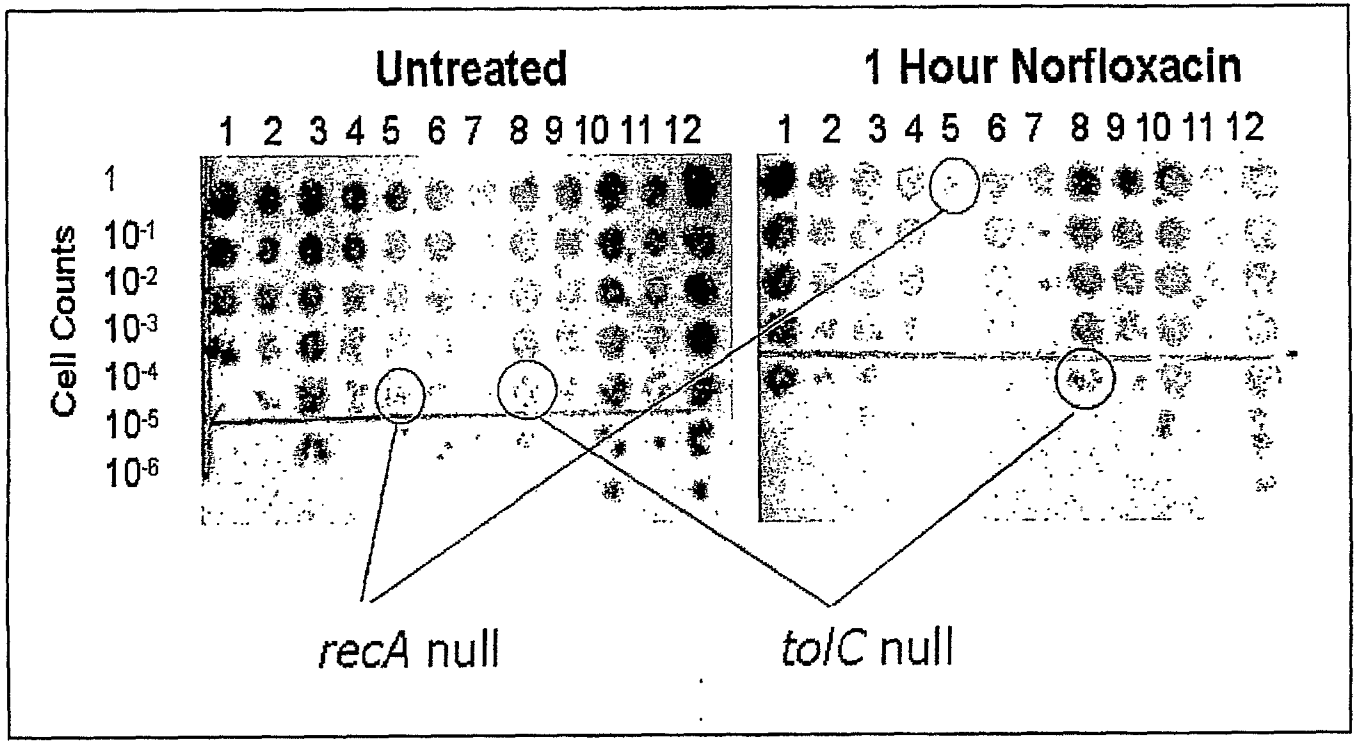

Untreated
1 Hour Norfloxacin
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5 6 7 8 9 10 11 12
Cell Counts
1
10-1
10-2
10-3
10-4
10-5
10-6
recA null
tolC null

Figure 2

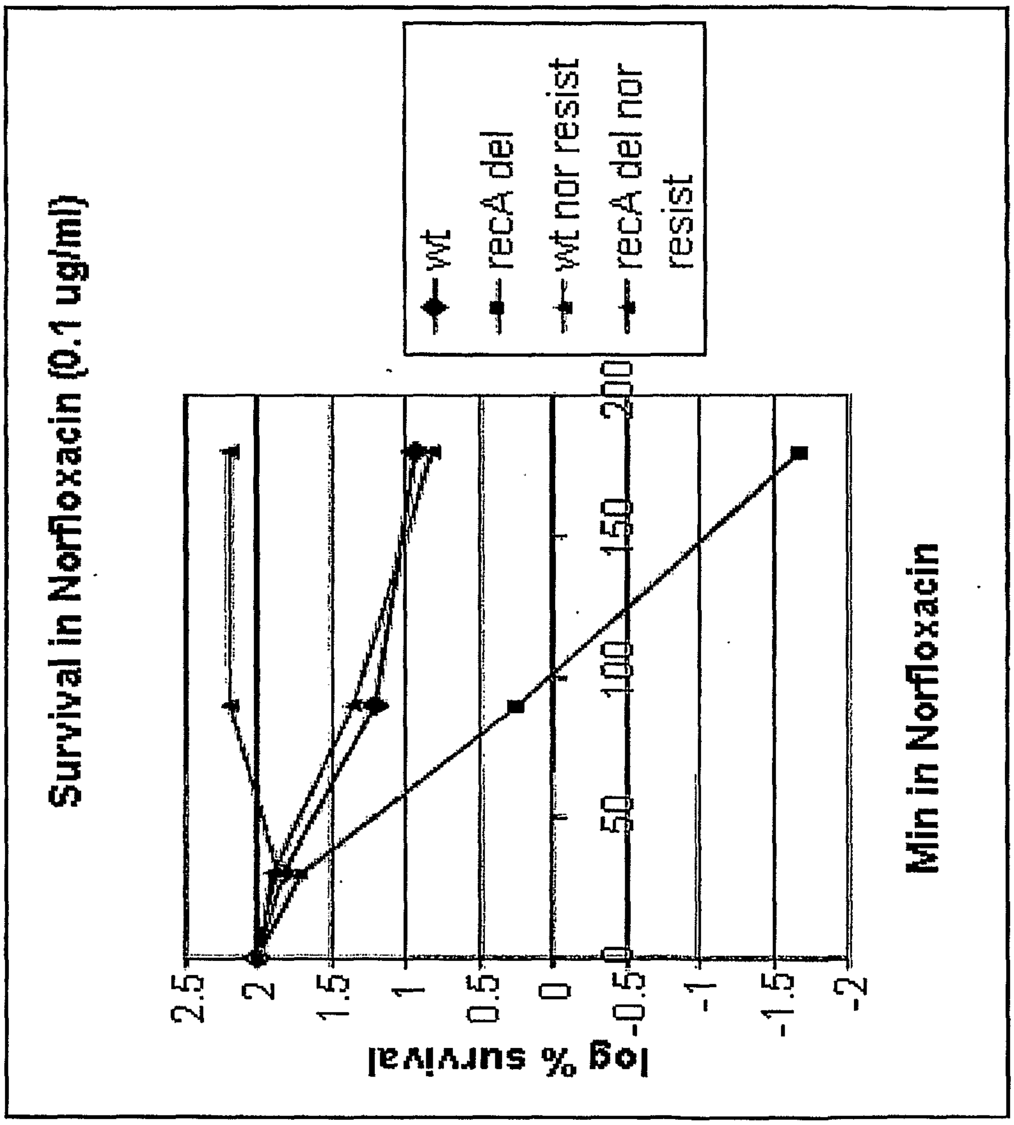

Survival in Norfloxacin (0.1 ug/ml)
log % survival
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
0
50
100
150
200
Min in Norfloxacin
wt
recA del
wt nor resist
recA del nor resist

Figure 3

E. coli parent, *recA* and *tolC* null strains in response to Norfloxacin using a growth and survival assay

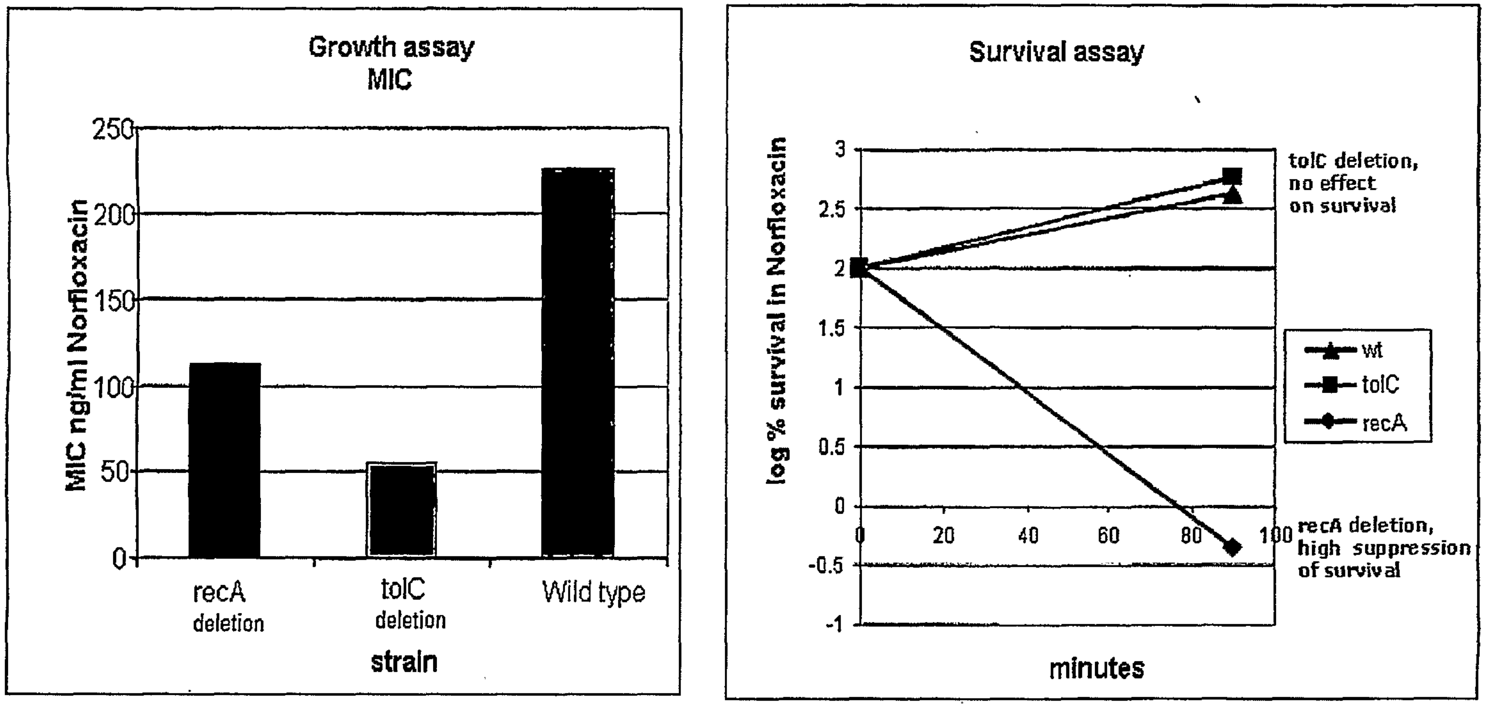


Figure 4

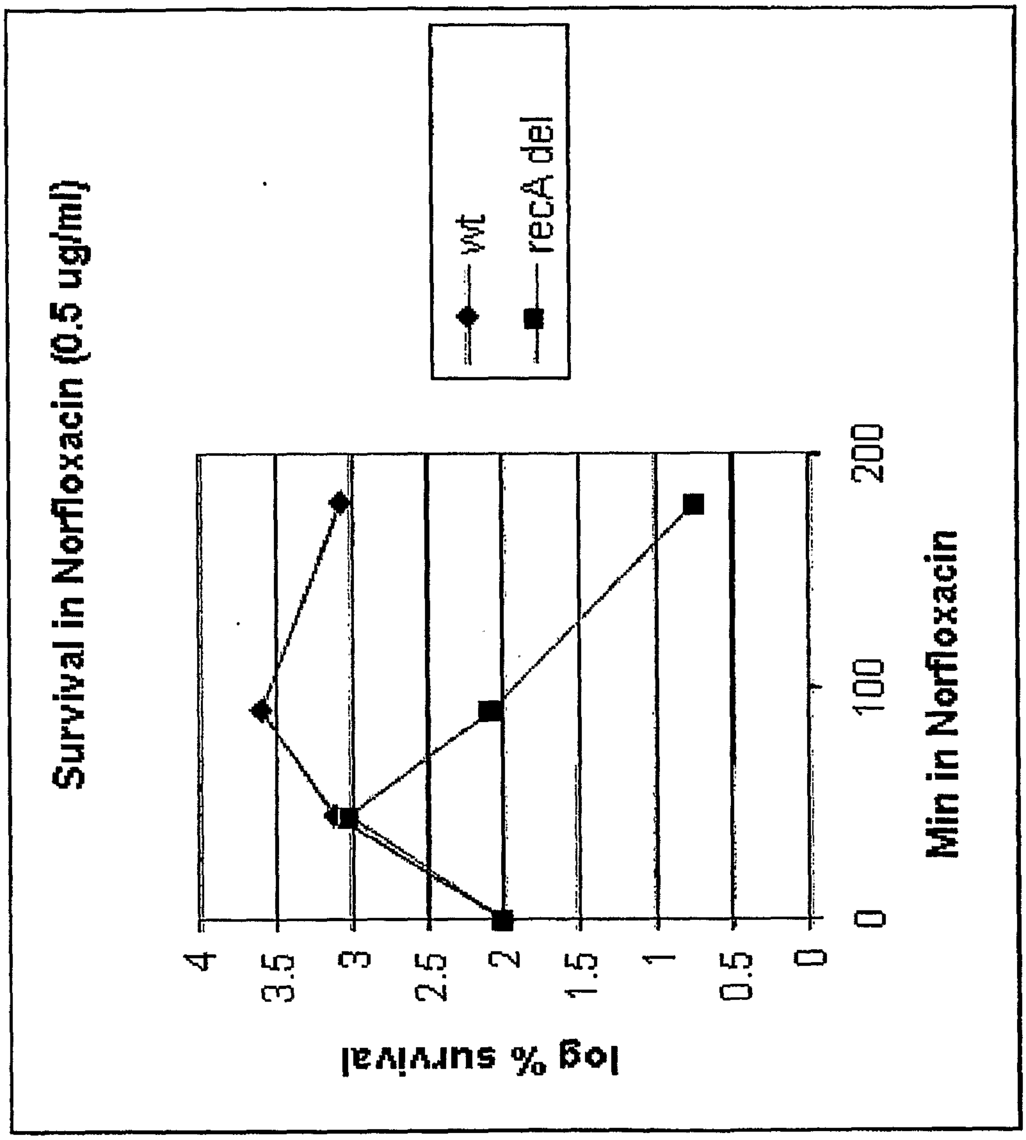

Survival in Norfloxacin (0.5 ug/ml)
wt
recA del
log % survival
4
3.5
3
2.5
2
1.5
1
0.5
0
0
100
200
Min in Norfloxacin

Figure 5

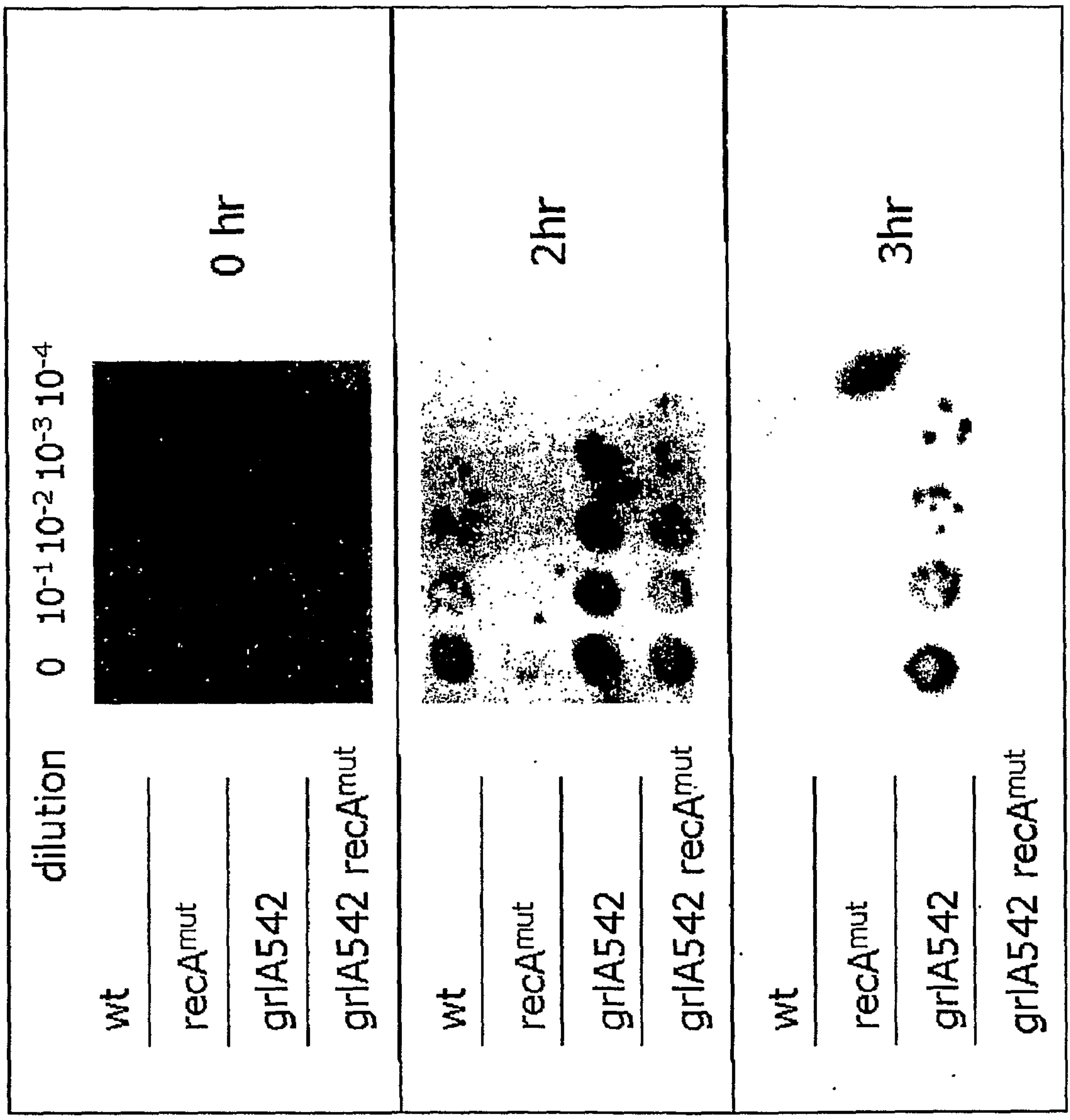

dilution
0 10-1 10-2 10-3 10-4
wt
recAmut
grlA542
grlA542 recAmut
0 hr
2hr
3hr

Figure 6

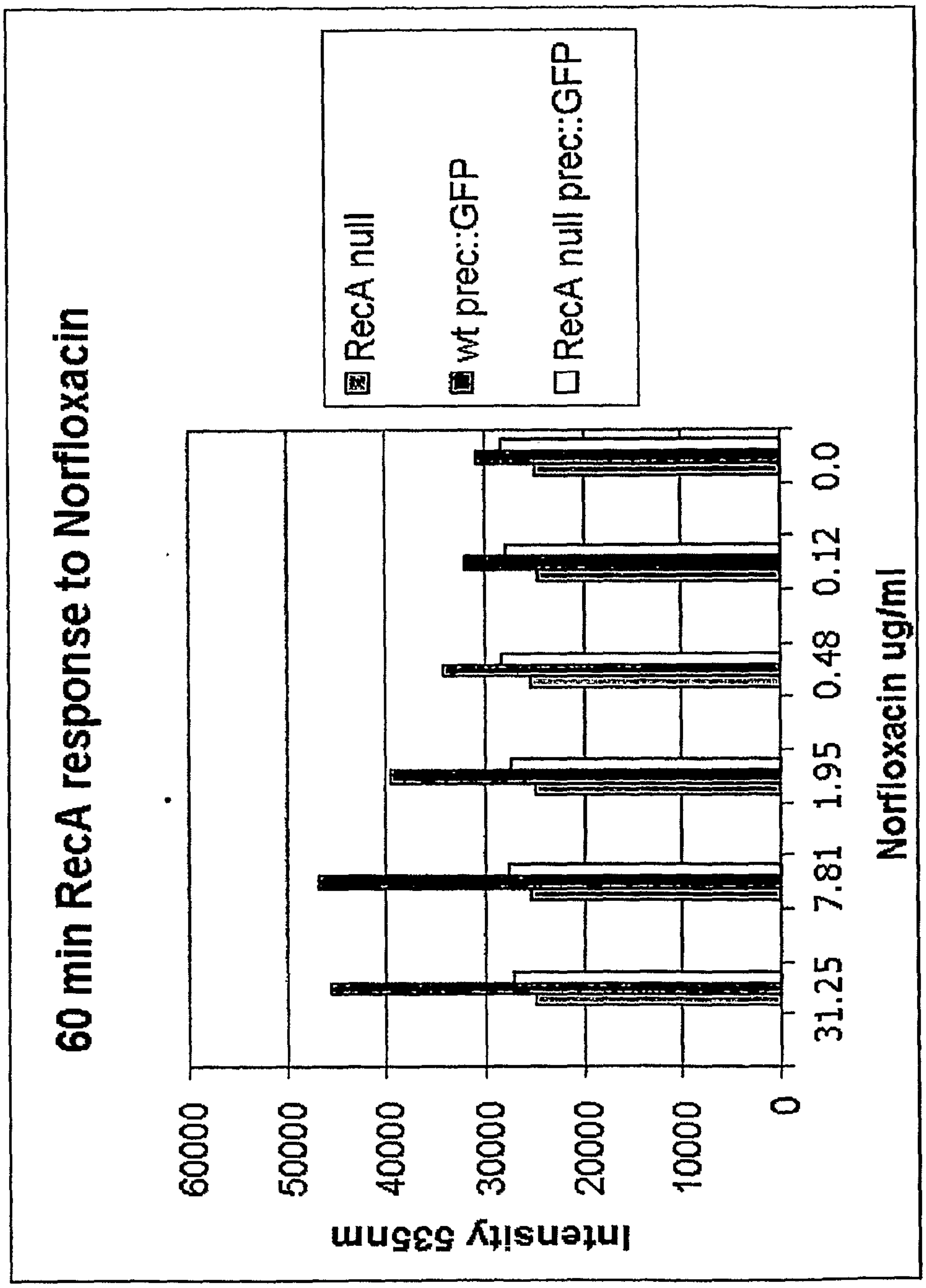

60 min RecA response to Norfloxacin
▨ RecA null
■ wt prec::GFP
☐ RecA null prec::GFP
Intensity 535nm
60000
50000
40000
30000
20000
10000
0
31.25
7.81
1.95
0.48
0.12
0.0
Norfloxacin ug/ml

Figure 7

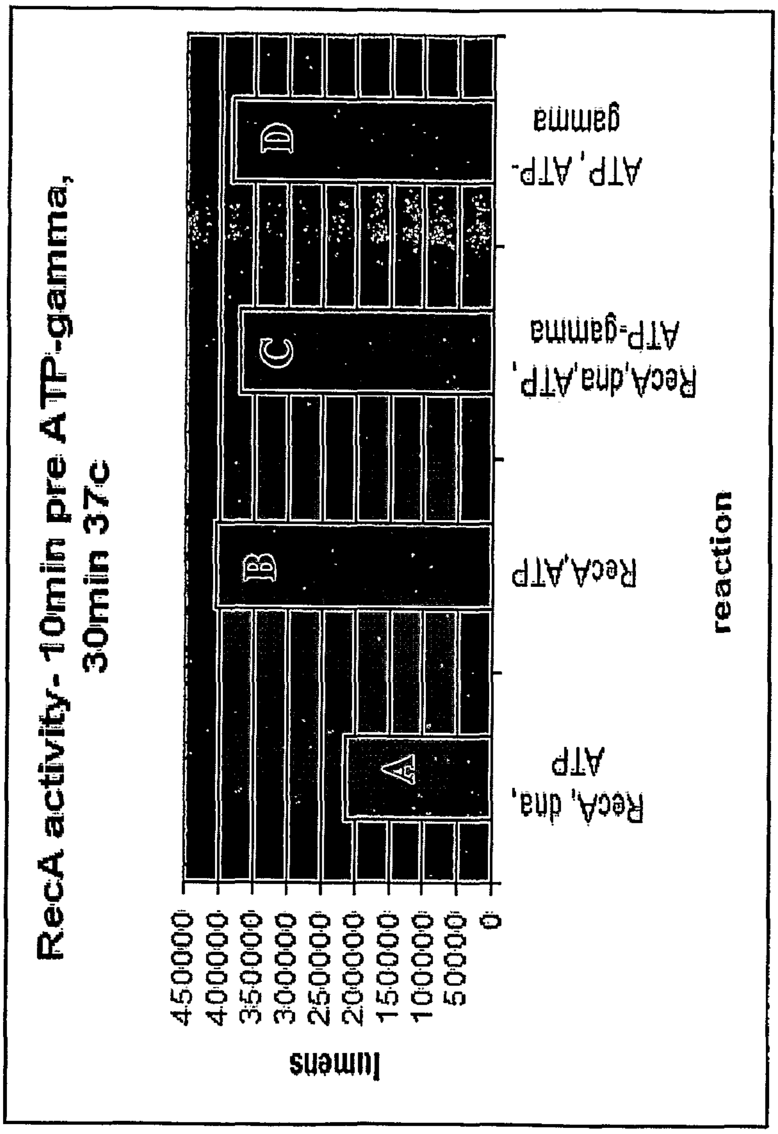
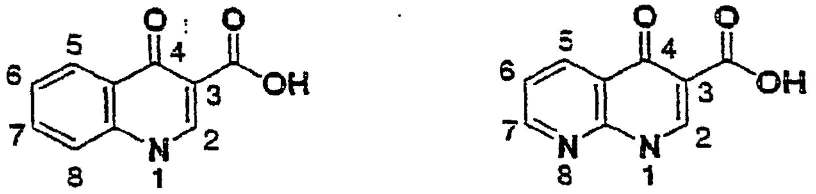

RecA activity- 10min pre ATP-gamma, 30min 37c
lumens
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
A
B
C
D
RecA, dna, ATP
RecA,ATP
RecA,dna,ATP, ATP-gamma
ATP, ATP-gamma
reaction

Figure 8



4-oxo-1,4-dihydroquinoline

4-oxo-1,4-dihydronaphthyridine

Figure 9

4-Quinolone

2-Pyridone

4-oxo-1,4-dihydroquinoline

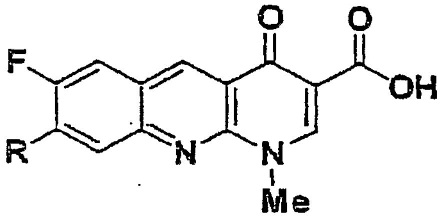
4*H*-4—oxoquinolizine

4-oxo-1,4-dihydronaphthyridine

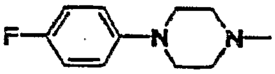
6*H*-6-oxo-pyrido[1,2-a]pyrimidine

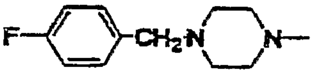
Figure 10

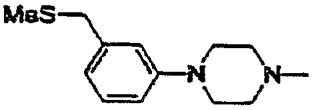
benzo[b]naphthyridones



R







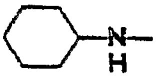
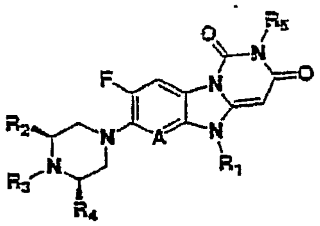
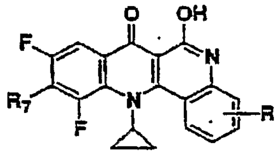


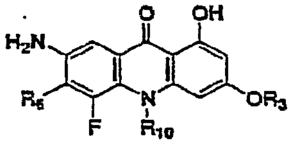
Figure 11 (A)



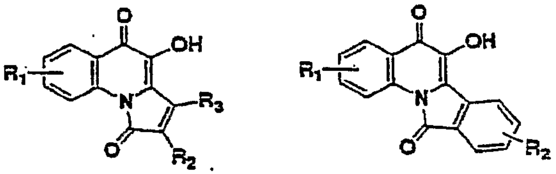
Pyrimido[1,6-a]benzimidazoles



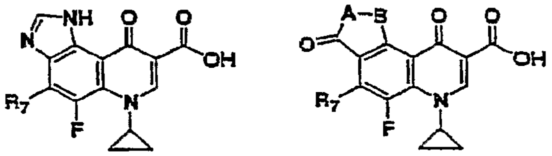
Dibenzo[1,6]naphthyridiones



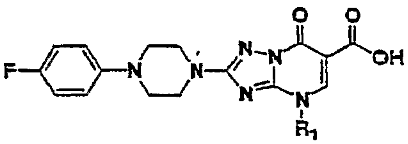
1-Hydroxy-7-amino-9-acridones



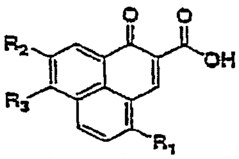
Pyrrolo[1,2-a]quinolines and Isoindolo[2,1-a]quinolines



Pyrroloquinolones and Pyrazoloquinolones



2-[4-(4-Fluorophenyl)piperizin-1-yl]-4,7-dihydro-7-oxo-1,2,4-triazolo[1,5-a]pyrimidine-6-carboxylic Acids



p-Quinone Methides

Figure 11 (B)

Conservation Query

GENE_ID _ECOLI	ECOCYC _ID_ ECOLI	K12_ GENE	SUBJECT_ORGANISM	SUBJECT_ID	%_IDENT	E_ VALUE	BIT_ SCORE
945108	EG11704	acrB	Escherichia_coli_K12	gi|1786667|gb|AAC73564.1|	100	0	1918
945108	EG11704	acrB	Listeria_monocytogenes	gi|1786667|gb|AAC73564.1|	100	0	1918
945108	EG11704	acrB	Pseudomonas_aeruginosa	gi|9946282|gb|AAG03815.1|AE004479_10	70.95	0	1368
945108	EG11704	acrB	Salmonella_typhimurium_LT2	gi|16418982|gb|AAL19429.1|	94.66	0	1830
945108	EG11704	acrB	Shigella_flexneri_2a	gi|24050639|gb|AAN42062.1|	99.52	0	1907
945108	EG11704	acrB	Yersinia_pestis_KIM	gi|21957797|gb|AAM84630.1|AE013708_9	84.15	0	1636
945112	EG11703	acrA	Escherichia_coli_K12	gi|1786668|gb|AAC73565.1|	100	0	680
945112	EG11703	acrA	Listeria_monocytogenes	gi|1786668|gb|AAC73565.1|	100	0	680
945112	EG11703	acrA	Salmonella_typhimurium_LT2	gi|16418983|gb|AAL19430.1|	91.69	0	626
945112	EG11703	acrA	Shigella_flexneri_2a	gi|56383219|gb|AAN42063.2|	99.5	0	678
948589	G6653	ycjS	Escherichia_coli_K12	gi|1787574|gb|AAC74397.1|	100	0	713
948589	G6653	ycjS	Listeria_monocytogenes	gi|1787574|gb|AAC74397.1|	100	0	713
946005	G6751	b1440	Escherichia_coli_K12	gi|1787711|gb|AAC74522.1|	100	0	800
946005	G6751	b1440	Listeria_monocytogenes	gi|1787711|gb|AAC74522.1|	100	0	800
945861	G6816	ydfI	Escherichia_coli_K12	gi|1787823|gb|AAC74615.1|	100	0	976
945861	G6816	ydfI	Listeria_monocytogenes	gi|1787823|gb|AAC74615.1|	100	0	976
945861	G6816	ydfI	Salmonella_typhimurium_LT2	gi|16420028|gb|AAL20427.1|	81.4	0	795
945861	G6816	ydfI	Shigella_flexneri_2a	gi|24051875|gb|AAN43142.1|	98.35	0	964
946371	EG10924	ruvB	Escherichia_coli_K12	gi|1788167|gb|AAC74930.1|	100	0	659
946371	EG10924	ruvB	Listeria_monocytogenes	gi|1788167|gb|AAC74930.1|	100	0	659
946371	EG10924	ruvB	Salmonella_typhimurium_LT2	gi|16420430|gb|AAL20810.1|	96.43	0	636
946371	EG10924	ruvB	Shigella_flexneri_2a	gi|24052206|gb|AAN43427.1|	99.7	0	657
946652	EG12002	yehP	Escherichia_coli_K12	gi|1788440|gb|AAC75182.1|	100	0	745
946652	EG12002	yehP	Listeria_monocytogenes	gi|1788440|gb|AAC75182.1|	100	0	745
946652	EG12002	yehP	Shigella_flexneri_2a	gi|56383592|gb|AAN43716.2|	98.94	0	734
946716	EG10670	ompC	Escherichia_coli_K12	gi|1788544|gb|AAC75275.1|	100	0	714
946716	EG10670	ompC	Listeria_monocytogenes	gi|1788544|gb|AAC75275.1|	100	0	714
946716	EG10670	ompC	Shigella_flexneri_2a	gi|24052654|gb|AAN43817.1|	91.78	0	661
946988	EG11072	xseA	Escherichia_coli_K12	gi|1788856|gb|AAC75562.1|	100	0	795
946988	EG11072	xseA	Listeria_monocytogenes	gi|1788856|gb|AAC75562.1|	100	0	795
946988	EG11072	xseA	Salmonella_typhimurium_LT2	gi|16421055|gb|AAL21406.1|	87.47	0	707
946988	EG11072	xseA	Shigella_flexneri_2a	gi|56383673|gb|AAN44055.2|	98.03	0	779
947170	EG10823	recA	Escherichia_coli_K12	gi|1789051|gb|AAC75741.1|	100	0	663
947170	EG10823	recA	Listeria_monocytogenes	gi|1789051|gb|AAC75741.1|	100	0	663

Figure 12 (page 1 of 11)

947170	EG10823	recA	Salmonella_typhimurium_LT2	gi|16421374|gb|AAL21709.1|	97.17	0	648
947170	EG10823	recA	Shigella_flexneri_2a	gi|56383715|gb|AAN44214.2|	100	0	663
947286	EG10824	recB	Escherichia_coli_K12	gi|1789183|gb|AAC75859.1|	100	0	2271
947286	EG10824	recB	Haemophilus_influenzae	gi|1574781|gb|AAC22966.1|	35.68	0	627
947286	EG10824	recB	Listeria_monocytogenes	gi|1789183|gb|AAC75859.1|	100	0	2271
947286	EG10824	recB	Pseudomonas_aeruginosa	gi|9950504|gb|AAG07672.1|AE004844_4	39.86	0	691
947286	EG10824	recB	Salmonella_typhimurium_LT2	gi|16421543|gb|AAL21870.1|	84.75	0	1937
947286	EG10824	recB	Shigella_flexneri_2a	gi|56383740|gb|AAN44318.2|	98.47	0	2240
947286	EG10824	recB	Vibrio_cholerae	gi|9656886|gb|AAF95464.1|	46.54	0	929
947286	EG10824	recB	Yersinia_pestis_KIM	gi|21960090|gb|AAM86714.1|AE013917_2	62.02	0	1414
947294	EG10825	recC	Escherichia_coli_K12	gi|1789186|gb|AAC75861.1|	100	0	2199
947294	EG10825	recC	Haemophilus_influenzae	gi|1573962|gb|AAC22596.1|	37.47	0	626
947294	EG10825	recC	Listeria_monocytogenes	gi|1789186|gb|AAC75861.1|	100	0	2199
947294	EG10825	recC	Salmonella_typhimurium_LT2	gi|16421545|gb|AAL21872.1|	88.5	0	1986
947294	EG10825	recC	Shigella_flexneri_2a	gi|24053236|gb|AAN44320.1|	99.64	0	2190
947294	EG10825	recC	Vibrio_cholerae	gi|9656888|gb|AAF95466.1|	44.7	0	884
947294	EG10825	recC	Yersinia_pestis_KIM	gi|21960092|gb|AAM86716.1|AE013917_4	63.4	0	1428
947521	EG11009	tolC	Escherichia_coli_K12	gi|1789413|gb|AAC76071.1|	100	0	889
947521	EG11009	tolC	Listeria_monocytogenes	gi|1789413|gb|AAC76071.1|	100	0	889
947521	EG11009	tolC	Salmonella_typhimurium_LT2	gi|16421742|gb|AAL22060.1|	89.7	0	791
947521	EG11009	tolC	Shigella_flexneri_2a	gi|56383795|gb|AAN44553.2|	99.8	0	883
948356	EG10746	polA	Escherichia_coli_K12	gi|1790294|gb|AAC76861.1|	100	0	1696
948356	EG10746	polA	Francisella_tularensis_tularensis	gi|56603777|emb|CAG44744.1|	52.33	0	836
948356	EG10746	polA	Haemophilus_influenzae	gi|1573871|gb|AAC22515.1|	62.78	0	1068
948356	EG10746	polA	Listeria_monocytogenes	gi|1790294|gb|AAC76861.1|	100	0	1696
948356	EG10746	polA	Pseudomonas_aeruginosa	gi|9951828|gb|AAG08878.1|AE004962_2	62.03	0	1038
948356	EG10746	polA	Salmonella_typhimurium_LT2	gi|16422560|gb|AAL22838.1|	93.86	0	1615
948356	EG10746	polA	Shigella_flexneri_2a	gi|24054427|gb|AAN45369.1|	99.46	0	1688
948356	EG10746	polA	Vibrio_cholerae	gi|9654507|gb|AAF93286.1|	64.67	0	1121
948356	EG10746	polA	Yersinia_pestis_KIM	gi|21960799|gb|AAM87356.1|AE013984_2	77.6	0	1322
948426	EG10763	priA	Escherichia_coli_K12	gi|1790370|gb|AAC76917.1|	100	0	1455
948426	EG10763	priA	Haemophilus_influenzae	gi|1573308|gb|AAC22001.1|	51.93	0	722
948426	EG10763	priA	Listeria_monocytogenes	gi|1790370|gb|AAC76917.1|	100	0	1455
948426	EG10763	priA	Pseudomonas_aeruginosa	gi|9951341|gb|AAG08435.1|AE004918_5	50.21	0	679
948426	EG10763	priA	Salmonella_typhimurium_LT2	gi|16422662|gb|AAL22935.1|	90.54	0	1355
948426	EG10763	priA	Shigella_flexneri_2a	gi|56384037|gb|AAN45446.2|	99.03	0	1441
948426	EG10763	priA	Vibrio_cholerae	gi|9657272|gb|AAF95819.1|	54.39	0	759

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<table>
<tr><td>948426</td><td>EG10763</td><td>priA</td><td>Yersinia_pestis_KIM</td><td>gi|21956978|gb|AAM83890.1|AE013629_1</td><td>73.16</td><td>0</td><td>1079</td></tr>
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<tr><td>948162</td><td>EG10829</td><td>recG</td><td>Salmonella_typhimurium_LT2</td><td>gi|16422313|gb|AAL22603.1|</td><td>94.37</td><td>0</td><td>1237</td></tr>
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<tr><td>948162</td><td>EG10829</td><td>recG</td><td>Yersinia_pestis_KIM</td><td>gi|21956768|gb|AAM83699.1|AE013610_11</td><td>81.53</td><td>0</td><td>1073</td></tr>
<tr><td>948347</td><td>EG11064</td><td>uvrD</td><td>Escherichia_coli_K12</td><td>gi|2367296|gb|AAC76816.1|</td><td>100</td><td>0</td><td>1393</td></tr>
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<tr><td>946003</td><td>EG10277</td><td>fabH</td><td>Escherichia_coli_K12</td><td>gi|1787333|gb|AAC74175.1|</td><td>100</td><td>1E-180</td><td>625</td></tr>
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<tr><td>946003</td><td>EG10277</td><td>fabH</td><td>Salmonella_typhimurium_LT2</td><td>gi|16419710|gb|AAL20122.1|</td><td>94.64</td><td>3E-172</td><td>597</td></tr>
<tr><td>947362</td><td>EG11071</td><td>xerD</td><td>Escherichia_coli_K12</td><td>gi|1789261|gb|AAC75932.1|</td><td>100</td><td>1E-170</td><td>592</td></tr>
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<tr><td>946988</td><td>EG11072</td><td>xseA</td><td>Yersinia_pestis_KIM</td><td>gi|21958131|gb|AAM84934.1|AE013738_11</td><td>71.81</td><td>6E-170</td><td>590</td></tr>
<tr><td>945484</td><td>EG10852</td><td>rimK</td><td>Escherichia_coli_K12</td><td>gi|1787076|gb|AAC73939.1|</td><td>100</td><td>5E-169</td><td>586</td></tr>
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947170	EG10823	recA	Yersinia_pestis_KIM	gi|21957615|gb|AAM84465.1|AE013691_4	87.92	2E-167	582
947105	EG10831	recN	Vibrio_cholerae	gi|9655304|gb|AAF94014.1|	56.24	2E-166	578
948347	EG11064	uvrD	Francisella_tularensis_tularensis	gi|56603787|emb|CAG44754.1|	42.51	3E-166	577
945484	EG10852	rimK	Shigella_flexneri_2a	gi|24051068|gb|AAN42439.1|	99	4E-166	577
947362 -	EG11071	xerD	Salmonella_typhimurium_LT2	gi|16421595|gb|AAL21919.1|	95.3	1E-163	568
946716	EG10670	ompC	Salmonella_typhimurium_LT2	gi|16420806|gb|AAL21169.1|	77.78	7E-162	563
945861	G6816	ydfI	Yersinia_pestis_KIM	gi|21959806|gb|AAM86454.1|AE013893_1	57.68	2E-161	562
946371	EG10924	ruvB	Yersinia_pestis_KIM	gi|21959099|gb|AAM85812.1|AE013828_3	84.73	2E-161	561
948356	EG10746	polA	Clostridium_difficile	CD1128	37.23	2E-161	563
949035	EG11002	thyA	Escherichia_coli_K12	gi|1789191|gb|AAC75866.1|	100	3E-161	560
949035	EG11002	thyA	Listeria_monocytogenes	gi|1789191|gb|AAC75866.1|	100	3E-161	560
949035	EG11002	thyA	Shigella_flexneri_2a	gi|24053240|gb|AAN44323.1|	99.62	3E-161	560
949035	EG11002	thyA	Salmonella_typhimurium_LT2	gi|16421551|gb|AAL21877.1|	96.21	7E-157	546
948356	EG10746	polA	Bacillus_anthracis_Ames	gi|30259332|gb|AAP28520.1|	37.53	2E-155	543
945484	EG10852	rimK	Salmonella_typhimurium_LT2	gi|16419383|gb|AAL19811.1|	90.33	4E-155	540
946371	EG10924	ruvB	Haemophilus_influenzae	gi|1573279|gb|AAC21975.1|	81.52	2E-153	533
947170	EG10823	recA	Vibrio_cholerae	gi|9654971|gb|AAF93711.1|	84.29	2E-153	534
948356	EG10746	polA	Mycobacterium_tuberculosis_CDC1551	gi|16410994|emb|CAC99643.1|	38.58	3E-152	532
948356	EG10746	polA	Staphylococcus_aureus_aureus_MRSA252	gi|49242061|emb|CAG40760.1|	37.78	5E-151	528
946371	EG10924	ruvB	Vibrio_cholerae	gi|9656373|gb|AAF94993.1|	80.42	5E-149	520
948347	EG11064	uvrD	Mycobacterium_tuberculosis_CDC1551	gi|16411213|emb|CAC99837.1|	41.19	8E-147	513
946003	EG10277	fabH	Yersinia_pestis_KIM	gi|21958561|gb|AAM85324.1|AE013778_10	81.07	1E-146	512
948364	EG10209	dapF	Escherichia_coli_K12	gi|1790242|gb|AAC76812.1|	100	8E-146	509
948364	EG10209	dapF	Listeria_monocytogenes	gi|1790242|gb|AAC76812.1|	100	8E-146	509
948347	EG11064	uvrD	Staphylococcus_aureus_aureus_MRSA252	gi|49242275|emb|CAG40982.1|	39.81	2E-145	508
948364	EG10209	dapF	Shigella_flexneri_2a	gi|24054374|gb|AAN45323.1|	99.64	3E-145	507
949035	EG11002	thyA	Yersinia_pestis_KIM	gi|21960098|gb|AAM86721.1|AE013918_5	88.64	6E-145	506
947170	EG10823	recA	Haemophilus_influenzae	gi|1573588|gb|AAC22257.1|	73.37	8E-145	505
946716	EG10670	ompC	Yersinia_pestis_KIM	gi|21959873|gb|AAM86517.1|AE013897_13	70.59	2E-144	505
947362	EG11071	xerD	Yersinia_pestis_KIM	gi|21960213|gb|AAM86826.1|AE013928_11	83.5	1E-143	502
945112	EG11703	acrA	Yersinia_pestis_KIM	gi|21957798|gb|AAM84631.1|AE013708_10	75.53	3E-142	498
948364	EG10209	dapF	Salmonella_typhimurium_LT2	gi|16422511|gb|AAL22791.1|	95.64	3E-142	497
948356	EG10746	polA	Clostridium_botulinum	CBO3014	36.5	1E-141	497
948347	EG11064	uvrD	Enterococcus_faecalis_V583	gi|29342777|gb|AAO80541.1|	39.44	5E-140	491
948356	EG10746	polA	Streptococcus_pneumoniae_R6	gi|15457562|gb|AAK98836.1|	36.79	1E-139	489
947105	EG10831	recN	Haemophilus_influenzae	gi|1573019|gb|AAC21748.1|	49.1	8E-139	486
946371	EG10924	ruvB	Francisella_tularensis_tularensis	gi|56604594|emb|CAG45646.1|	72.62	1E-138	484

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948347	EG11064	uvrD	Bacillus_anthracis_Ames	gi|30253845|gb|AAP24340.1|	39.19	2E-138	486
946371	EG10924	ruvB	Pseudomonas_aeruginosa	gi|9946874|gb|AAG04356.1|AE004530_9	72.24	6E-136	477
948347	EG11064	uvrD	Streptococcus_pneumoniae_R6	gi|15458610|gb|AAK99799.1|	37.68	1E-134	472
947170	EG10823	recA	Pseudomonas_aeruginosa	gi|9949775|gb|AAG07005.1|AE004782_3	71.14	3E-134	472
945108	EG11704	acrB	Vibrio_cholerae	gi|9654566|gb|AAF93340.1|	32.16	3E-133	469
946003	EG10277	fabH	Vibrio_cholerae	gi|9656567|gb|AAF95171.1|	72.56	1E-132	465
948347	EG11064	uvrD	Clostridium_botulinum	CBO3271	36.81	1E-132	466
948347	EG11064	uvrD	Clostridium_difficile	CD0328	37.62	2E-132	466
945108	EG11704	acrB	Haemophilus_influenzae	gi|1573914|gb|AAC22555.1|	32.34	6E-131	461
946988	EG11072	xseA	Haemophilus_influenzae	gi|1573368|gb|AAC22056.1|	57.44	3E-127	447
948364	EG10209	dapF	Yersinia_pestis_KIM	gi|21957072|gb|AAM83974.1|AE013639_3	85.04	2E-125	441
946988	EG11072	xseA	Vibrio_cholerae	gi|9655213|gb|AAF93931.1|	56.88	7E-125	440
947170	EG10823	recA	Francisella_tularensis_tularensis	gi|56605240|emb|CAG46383.1|	65.69	7E-124	435
947105	EG10831	recN	Pseudomonas_aeruginosa	gi|9951027|gb|AAG08149.1|AE004890_2	44.2	8E-123	434
948426	EG10763	priA	Francisella_tularensis_tularensis	gi|56603868|emb|CAG44848.1|	37.09	1E-120	426
945108	EG11704	acrB	Francisella_tularensis_tularensis	gi|56603771|emb|CAG44738.1|	28.4	2E-120	426
947170	EG10823	recA	Clostridium_difficile	CD1328	64.31	4E-120	424
946371	EG10924	ruvB	Bacillus_anthracis_Ames	gi|30259148|gb|AAP28353.1|	60.73	2E-119	422
947170	EG10823	recA	Clostridium_botulinum	CBO2405	66.05	2E-119	421
947362	EG11071	xerD	Haemophilus_influenzae	gi|1573278|gb|AAC21974.1|	69.15	2E-119	421
948162	EG10829	recG	Francisella_tularensis_tularensis	gi|56605113|emb|CAG46234.1|	39.12	1E-117	416
946003	EG10277	fabH	Haemophilus_influenzae	gi|1573114|gb|AAC21826.1|	65.93	2E-117	414
948162	EG10829	recG	Clostridium_difficile	CD2560	41.43	5E-117	415
948426	EG10763	priA	Enterococcus_faecalis_V583	gi|29345047|gb|AAO82804.1|	34.05	7E-117	414
947362	EG11071	xerD	Vibrio_cholerae	gi|9656993|gb|AAF95562.1|	69.46	9E-117	412
946371	EG10924	ruvB	Clostridium_difficile	CD2805	60.42	2E-116	411
948426	EG10763	priA	Staphylococcus_aureus_aureus_MRSA252	gi|49241504|emb|CAG40190.1|	39.02	5E-116	411
946371	EG10924	ruvB	Mycobacterium_tuberculosis_CDC1551	gi|16410961|emb|CAC99610.1|	60.37	9E-116	409
949035	EG11002	thyA	Pseudomonas_aeruginosa	gi|9946191|gb|AAG03731.1|AE004472_3	69.7	2E-115	409
948364	EG10209	dapF	Haemophilus_influenzae	gi|1573758|gb|AAC22409.1|	77.37	1E-114	404
945484	EG10852	rimK	Vibrio_cholerae	gi|9656844|gb|AAF95425.1|	68.26	2E-114	404
946371	EG10924	ruvB	Enterococcus_faecalis_V583	gi|29342181|gb|AAO79947.1|	59.27	9E-114	402
948426	EG10763	priA	Mycobacterium_tuberculosis_CDC1551	gi|16411278|emb|CAC99902.1|	38.33	1E-113	403
947170	EG10823	recA	Staphylococcus_aureus_aureus_MRSA252	gi|49241577|emb|CAG40263.1|	62.58	2E-113	401
946371	EG10924	ruvB	Clostridium_botulinum	CBO3070	61.76	3E-113	401
948426	EG10763	priA	Bacillus_anthracis_Ames	gi|30258515|gb|AAP27734.1|	39.35	5E-113	402
946371	EG10924	ruvB	Staphylococcus_aureus_aureus_MRSA252	gi|49242014|emb|CAG40712.1|	60.19	6E-113	399

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948364	EG10209	dapF	Vibrio_cholerae	gi|9654526|gb|AAF93303.1|	78.52	9E-113	399
947170	EG10823	recA	Mycobacterium_tuberculosis_CDC1551	gi|16410827|emb|CAC99476.1|	63.86	3E-112	397
947170	EG10823	recA	Enterococcus_faecalis_V583	gi|29345089|gb|AAO82845.1|	59.88	9E-112	396
947170	EG10823	recA	Bacillus_anthracis_Ames	gi|30258429|gb|AAP27648.1|	62.93	2E-111	395
948426	EG10763	priA	Streptococcus_pneumoniae_R6	gi|15459247|gb|AAL00384.1|	34.22	2E-111	395
945484	EG10852	rimK	Pseudomonas_aeruginosa	gi|9951502|gb|AAG08582.1|AE004932_9	67.82	4E-110	391
948589	G6653	ycjS	Mycobacterium_tuberculosis_CDC1551	gi|16412334|emb|CAD01047.1|	57.02	6E-110	390
947286	EG10824	recB	Francisella_tularensis_tularensis	gi|56604935|emb|CAG46027.1|	27.15	2E-109	389
946371	EG10924	ruvB	Streptococcus_pneumoniae_R6	gi|15457786|gb|AAK99042.1|	59.55	2E-108	384
948356	EG10746	polA	Enterococcus_faecalis_V583	gi|29342923|gb|AAO80687.1|	44.44	6E-108	385
946088	G6819	b1545	Escherichia_coli_K12	gi|1787827|gb|AAC74618.1|	100	2E-107	381
946088	G6819	b1545	Listeria_monocytogenes	gi|1787827|gb|AAC74618.1|	100	2E-107	381
948162	EG10829	recG	Mycobacterium_tuberculosis_CDC1551	gi|16411265|emb|CAC99889.1|	38.87	2E-107	383
948162	EG10829	recG	Enterococcus_faecalis_V583	gi|29345036|gb|AAO82793.1|	38.63	8E-107	380
947053	EG12341	rseA	Escherichia_coli_K12	gi|1788925|gb|AAC75625.1|	100	2E-106	377
947053	EG12341	rseA	Listeria_monocytogenes	gi|1788925|gb|AAC75625.1|	100	2E-106	377
947170	EG10823	recA	Streptococcus_pneumoniae_R6	gi|15459438|gb|AAL00560.1|	60	2E-106	378
948162	EG10829	recG	Streptococcus_pneumoniae_R6	gi|15459203|gb|AAL00343.1|	38.78	1E-105	376
947053	EG12341	rseA	Shigella_flexneri_2a	gi|56383693|gb|AAN44131.2|	99.54	2E-105	374
948426	EG10763	priA	Clostridium_difficile	CD2586	37.18	3E-103	369
948426	EG10763	priA	Clostridium_botulinum	CBO2511	32.76	7E-103	368
946369	EG10923	ruvA	Escherichia_coli_K12	gi|1788168|gb|AAC74931.1|	100	1E-102	365
946369	EG10923	ruvA	Listeria_monocytogenes	gi|1788168|gb|AAC74931.1|	100	1E-102	365
948162	EG10829	recG	Bacillus_anthracis_Ames	gi|30258502|gb|AAP27721.1|	39.91	1E-102	367
946369	EG10923	ruvA	Shigella_flexneri_2a	gi|24052207|gb|AAN43428.1|	99.01	1E-101	362
945112	EG11703	acrA	Pseudomonas_aeruginosa	gi|9946281|gb|AAG03814.1|AE004479_9	56.58	3E-101	362
947362	EG11071	xerD	Pseudomonas_aeruginosa	gi|9949906|gb|AAG07125.1|AE004793_2	63.79	1E-99	357
946369	EG10923	ruvA	Salmonella_typhimurium_LT2	gi|16420431|gb|AAL20811.1|	95.07	2E-99	354
948162	EG10829	recG	Clostridium_botulinum	CBO2496	36.6	3E-99	355
947294	EG10825	recC	Francisella_tularensis_tularensis	gi|56604938|emb|CAG46030.1|	27.74	2E-98	353
949035	EG11002	thyA	Francisella_tularensis_tularensis	gi|56604783|emb|CAG45862.1|	58.84	1E-96	344
947521	EG11009	tolC	Vibrio_cholerae	gi|9657012|gb|AAF95579.1|	46.83	3E-96	345
947053	EG12341	rseA	Salmonella_typhimurium_LT2	gi|16421188|gb|AAL21533.1|	91.67	4E-95	340
948162	EG10829	recG	Staphylococcus_aureus_aureus_MRSA252	gi|49241519|emb|CAG40205.1|	37.27	7E-94	337
946988	EG11072	xseA	Bacillus_anthracis_Ames	gi|30258900|gb|AAP28118.1|	42.92	9E-94	337
945861	G6816	ydfI	Pseudomonas_aeruginosa	gi|9948378|gb|AAG05730.1|AE004660_6	39.79	1E-93	337
947105	EG10831	recN	Enterococcus_faecalis_V583	gi|29343027|gb|AAO80790.1|	36.49	2E-93	336

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947105	EG10831	recN	Francisella_tularensis_tularensis	gi|56604479|emb|CAG45519.1|	36.59	3E-93	334
949035	EG11002	thyA	Bacillus_anthracis_Ames	gi|30256881|gb|AAP26113.1|	51.6	2E-92	332
949035	EG11002	thyA	Mycobacterium_tuberculosis_CDC1551	gi|16411328|emb|CAC99952.1|	50	7E-92	329
946988	EG11072	xseA	Pseudomonas_aeruginosa	gi|9949948|gb|AAG07164.1|AE004796_9	44.62	3E-89	323
949035	EG11002	thyA	Enterococcus_faecalis_V583	gi|29343601|gb|AAO81363.1|	51.77	2E-88	318
949035	EG11002	thyA	Staphylococcus_aureus_aureus_MRSA252	gi|49241747|emb|CAG40437.1|	51.13	6E-87	313
947105	EG10831	recN	Streptococcus_pneumoniae_R6	gi|15458707|gb|AAK99887.1|	32.73	9E-83	300
946378	EG10925	ruvC	Escherichia_coli_K12	gi|1788170|gb|AAC74933.1|	100	1E-82	298
946378	EG10925	ruvC	Listeria_monocytogenes	gi|1788170|gb|AAC74933.1|	100	1E-82	298
946003	EG10277	fabH	Francisella_tularensis_tularensis	gi|56604918|emb|CAG46006.1|	46.89	5E-82	296
948813	EG10244	dnaT	Shigella_flexneri_2a	gi|56384152|gb|AAN45808.2|	100	6E-82	296
948813	EG10244	dnaT	Escherichia_coli_K12	gi|1790824|gb|AAC77318.1|	100	7E-82	296
948813	EG10244	dnaT	Listeria_monocytogenes	gi|1790824|gb|AAC77318.1|	100	7E-82	296
946378	EG10925	ruvC	Shigella_flexneri_2a	gi|56383528|gb|AAN43430.2|	98.84	8E-82	295
946378	EG10925	ruvC	Salmonella_typhimurium_LT2	gi|16420434|gb|AAL20814.1|	98.27	9E-82	295
947362	EG11071	xerD	Francisella_tularensis_tularensis	gi|56603816|emb|CAG44787.1|	49.31	2E-81	294
946369	EG10923	ruvA	Yersinia_pestis_KIM	gi|21959100|gb|AAM85813.1|AE013828_4	79.02	3E-81	294
948364	EG10209	dapF	Pseudomonas_aeruginosa	gi|9951591|gb|AAG08663.1|AE004940_7	56	2E-80	293
946003	EG10277	fabH	Clostridium_difficile	CD1179	45.31	1E-78	286
946988	EG11072	xseA	Enterococcus_faecalis_V583	gi|29343022|gb|AAO80785.1|	38.92	2E-78	285
947105	EG10831	recN	Bacillus_anthracis_Ames	gi|30258894|gb|AAP28112.1|	32.69	1E-77	284
947105	EG10831	recN	Mycobacterium_tuberculosis_CDC1551	gi|16410784|emb|CAC99446.1|	32.98	3E-76	278
947105	EG10831	recN	Staphylococcus_aureus_aureus_MRSA252	gi|49241898|emb|CAG40592.1|	34.05	4E-76	278
944850	EG10230	dksA	Shigella_flexneri_2a	gi|56383163|gb|AAN41800.2|	100	7E-75	272
944850	EG10230	dksA	Escherichia_coli_K12	gi|1786338|gb|AAC73256.1|	100	8E-75	272
944850	EG10230	dksA	Listeria_monocytogenes	gi|1786338|gb|AAC73256.1|	100	8E-75	272
947105	EG10831	recN	Clostridium_difficile	CD1209	31.66	1E-74	273
947105	EG10831	recN	Clostridium_botulinum	CBO1877	31.66	2E-74	273
946988	EG11072	xseA	Mycobacterium_tuberculosis_CDC1551	gi|16410777|emb|CAC99439.1|	40.1	3E-74	271
944850	EG10230	dksA	Salmonella_typhimurium_LT2	gi|16418688|gb|AAL19150.1|	98.68	4E-74	270
946003	EG10277	fabH	Bacillus_anthracis_Ames	gi|30254898|gb|AAP25147.1|	45.28	2E-73	269
946423	EG10324	fliN	Escherichia_coli_K12	gi|1788256|gb|AAC75013.1|	100	5E-73	266
946423	EG10324	fliN	Listeria_monocytogenes	gi|1788256|gb|AAC75013.1|	100	5E-73	266
946988	EG11072	xseA	Streptococcus_pneumoniae_R6	gi|15458712|gb|AAK99892.1|	36.2	9E-73	266
946423	EG10324	fliN	Shigella_flexneri_2a	gi|24052332|gb|AAN43538.1|	97.81	1E-71	261
946003	EG10277	fabH	Mycobacterium_tuberculosis_CDC1551	gi|16411672|emb|CAD00280.1|	43.71	5E-71	260
944850	EG10230	dksA	Yersinia_pestis_KIM	gi|21957518|gb|AAM84378.1|AE013681_8	94.7	1E-70	258

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946378	EG10925	ruvC	Yersinia_pestis_KIM	gi|21959101|gb|AAM85814.1|AE013828_5	83.82	2E-69	254
946988	EG11072	xseA	Staphylococcus_aureus_aureus_MRSA252	gi|49241902|emb|CAG40596.1|	34.47	4E-69	254
948813	EG10244	dnaT	Salmonella_typhimurium_LT2	gi|16423112|gb|AAL23362.1|	81.56	5E-69	253
945484	EG10852	rimK	Haemophilus_influenzae	gi|1574372|gb|AAC23177.1|	44.63	2E-68	251
946369	EG10923	ruvA	Vibrio_cholerae	gi|9656374|gb|AAF94994.1|	64.85	5E-68	249
947362	EG11071	xerD	Bacillus_anthracis_Ames	gi|30258812|gb|AAP28030.1|	44.9	2E-67	249
946988	EG11072	xseA	Clostridium_difficile	CD1203	32.72	3E-67	249
946423	EG10324	fliN	Salmonella_typhimurium_LT2	gi|16420512|gb|AAL20889.1|	89.78	5E-67	246
947053	EG12341	rseA	Yersinia_pestis_KIM	gi|21958055|gb|AAM84865.1|AE013731_10	63.3	8E-67	246
946988	EG11072	xseA	Clostridium_botulinum	CBO1885	43.17	2E-66	246
947362	EG11071	xerD	Staphylococcus_aureus_aureus_MRSA252	gi|49241876|emb|CAG40569.1|	44.18	2E-66	244
946003	EG10277	fabH	Staphylococcus_aureus_aureus_MRSA252	gi|49241273|emb|CAG39952.1|	40.25	4E-66	244
947362	EG11071	xerD	Mycobacterium_tuberculosis_CDC1551	gi|16411408|emb|CAD00033.1|	45.92	5E-64	237
946003	EG10277	fabH	Streptococcus_pneumoniae_R6	gi|15457938|gb|AAK99181.1|	38.94	2E-63	234
946369	EG10923	ruvA	Haemophilus_influenzae	gi|1573280|gb|AAC21976.1|	59.31	4E-63	233
947362	EG11071	xerD	Enterococcus_faecalis_V583	gi|29343563|gb|AAO81325.1|	40.82	5E-63	234
946003	EG10277	fabH	Clostridium_botulinum	CBO3604	39.81	1E-62	233
947521	EG11009	tolC	Pseudomonas_aeruginosa	gi|9951257|gb|AAG08359.1|AE004910_6	34.25	9E-62	231
944850	EG10230	dksA	Vibrio_cholerae	gi|9655027|gb|AAF93763.1|	85.42	1E-61	228
945108	EG11704	acrB	Clostridium_difficile	CD2407	23.01	1E-60	228
946003	EG10277	fabH	Enterococcus_faecalis_V583	gi|29344818|gb|AAO82575.1|	39.76	3E-60	224
948589	G6653	ycjS	Staphylococcus_aureus_aureus_MRSA252	gi|49240579|emb|CAG39236.1|	36.97	3E-60	224
946378	EG10925	ruvC	Haemophilus_influenzae	gi|1573284|gb|AAC21978.1|	78.53	3E-59	219
945108	EG11704	acrB	Bacillus_anthracis_Ames	gi|30260133|gb|AAP29318.1|	22.93	2E-58	221
946003	EG10277	fabH	Pseudomonas_aeruginosa	gi|9949464|gb|AAG06721.1|AE004755_8	39.81	3E-58	219
946378	EG10925	ruvC	Vibrio_cholerae	gi|9656375|gb|AAF94995.1|	71.68	2E-56	211
946369	EG10923	ruvA	Pseudomonas_aeruginosa	gi|9946873|gb|AAG04355.1|AE004530_8	55.5	3E-54	205
945108	EG11704	acrB	Staphylococcus_aureus_aureus_MRSA252	gi|49242605|emb|CAG41326.1|	21.87	4E-53	202
944850	EG10230	dksA	Haemophilus_influenzae	gi|1573010|gb|AAC21740.1|	75.35	1E-52	197
947362	EG11071	xerD	Clostridium_botulinum	CBO1865	38.36	2E-52	199
947697	EG10317	fis	Shigella_flexneri_2a	gi|24053738|gb|AAN44763.1|	100	1E-51	193
947697	EG10317	fis	Escherichia_coli_K12	gi|1789661|gb|AAC76293.1|	100	2E-51	193
947697	EG10317	fis	Listeria_monocytogenes	gi|1789661|gb|AAC76293.1|	100	2E-51	193
947697	EG10317	fis	Salmonella_typhimurium_LT2	gi|16421946|gb|AAL22254.1|	100	2E-51	193
947697	EG10317	fis	Yersinia_pestis_KIM	gi|21956888|gb|AAM83808.1|AE013621_8	97.96	1E-50	190
946423	EG10324	fliN	Yersinia_pestis_KIM	gi|21959352|gb|AAM86041.1|AE013852_3	72.46	3E-49	187
945120	G7433	ygcO	Escherichia_coli_K12	gi|1789126|gb|AAC75809.1|	100	6E-49	185

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945120	G7433	ygcO	Listeria_monocytogenes	gi|1789126|gb|AAC75809.1|	100	6E-49	185
944850	EG10230	dksA	Pseudomonas_aeruginosa	gi|9950983|gb|AAG08109.1|AE004886_1	76.34	1E-48	185
945120	G7433	ygcO	Shigella_flexneri_2a	gi|56383731|gb|AAN44272.2|	91.86	5E-45	172
947362	EG11071	xerD	Clostridium_difficile	CD1222	33.33	5E-44	171
947697	EG10317	fis	Vibrio_cholerae	gi|9654701|gb|AAF93464.1|	82.65	3E-43	165
947697	EG10317	fis	Haemophilus_influenzae	gi|1574008|gb|AAC22640.1|	81.05	4E-41	157
946369	EG10923	ruvA	Francisella_tularensis_tularensis	gi|56604271|emb|CAG45291.1|	40.57	1E-40	158
945069	EG11098	xseB	Escherichia_coli_K12	gi|1786624|gb|AAC73525.1|	100	2E-40	156
945069	EG11098	xseB	Listeria_monocytogenes	gi|1786624|gb|AAC73525.1|	100	2E-40	156
945069	EG11098	xseB	Shigella_flexneri_2a	gi|24050588|gb|AAN42017.1|	100	2E-40	156
946378	EG10925	ruvC	Pseudomonas_aeruginosa	gi|9946872|gb|AAG04354.1|AE004530_7	55.49	4E-39	154
949035	EG11002	thyA	Haemophilus_influenzae	gi|1573924|gb|AAC22564.1|	33.92	7E-39	152
948356	EG10746	polA	Enterococcus_faecalis_V583	gi|29342923|gb|AAO80687.1|	34.13	1E-38	155
947521	EG11009	tolC	Francisella_tularensis_tularensis	gi|56605222|emb|CAG46357.1|	28	2E-37	149
949035	EG11002	thyA	Vibrio_cholerae	gi|9655113|gb|AAF93840.1|	32.16	8E-37	146
948364	EG10209	dapF	Bacillus_anthracis_Ames	gi|30259654|gb|AAP28840.1|	37.32	4E-36	145
945069	EG11098	xseB	Salmonella_typhimurium_LT2	gi|16418929|gb|AAL19378.1|	90	4E-35	139
947053	EG12341	rseA	Vibrio_cholerae	gi|9657044|gb|AAF95608.1|	45.18	6E-35	140
946378	EG10925	ruvC	Francisella_tularensis_tularensis	gi|56604270|emb|CAG45289.1|	54.09	9E-35	138
947053	EG12341	rseA	Haemophilus_influenzae	gi|1573625|gb|AAC22289.1|	40.74	1E-33	135
948364	EG10209	dapF	Clostridium_difficile	CD2590	33.1	4E-32	131
945069	EG11098	xseB	Yersinia_pestis_KIM	gi|21957754|gb|AAM84591.1|AE013704_8	77.38	2E-29	120
948364	EG10209	dapF	Clostridium_botulinum	CBO1912	33.81	1E-28	119
945052	G7522	yqgC	Escherichia_coli_K12	gi|1789309|gb|AAC75977.1|	100	4E-28	115
945052	G7522	yqgC	Listeria_monocytogenes	gi|1789309|gb|AAC75977.1|	100	4E-28	115
946423	EG10324	fliN	Pseudomonas_aeruginosa	gi|9947395|gb|AAG04833.1|AE004574_4	42.36	4E-27	114
946369	EG10923	ruvA	Bacillus_anthracis_Ames	gi|30259149|gb|AAP28354.1|	36.32	2E-26	112
946088	G6819	b1545	Staphylococcus_aureus_aureus_MRSA252	gi|49242119|emb|CAG40819.1|	35.83	4E-26	110
947286	EG10824	recB	Bacillus_anthracis_Ames	gi|30254863|gb|AAP25112.1|	23.02	1E-25	112
946369	EG10923	ruvA	Staphylococcus_aureus_aureus_MRSA252	gi|49242015|emb|CAG40713.1|	35.47	2E-25	108
946088	G6819	b1545	Salmonella_typhimurium_LT2	gi|16421248|gb|AAL21589.1|	37.22	7E-25	107
946088	G6819	b1545	Shigella_flexneri_2a	gi|56383689|gb|AAN44106.2|	42.11	1E-24	106
946423	EG10324	fliN	Vibrio_cholerae	gi|9656675|gb|AAF95270.1|	57.32	1E-24	104
949035	EG11002	thyA	Streptococcus_pneumoniae_R6	gi|15458166|gb|AAK99389.1|	30.8	7E-24	103
946378	EG10925	ruvC	Clostridium_difficile	CD2807	40.31	1E-23	102
948589	G6653	ycjS	Yersinia_pestis_KIM	gi|21959281|gb|AAM85976.1|AE013846_7	26.23	4E-23	102
946369	EG10923	ruvA	Streptococcus_pneumoniae_R6	gi|15457707|gb|AAK98969.1|	35	4E-23	100

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946369	EG10923	ruvA	Enterococcus_faecalis_V583	gi|29342180|gb|AAO79946.1|	33.86	1E-22	99
946369	EG10923	ruvA	Mycobacterium_tuberculosis_CDC1551	gi|16410962|emb|CAC99611.1|	32.84	2E-22	98.2
946369	EG10923	ruvA	Clostridium_botulinum	CBO3071	33.67	3E-22	97.8
946369	EG10923	ruvA	Clostridium_difficile	CD2806	33.17	5E-22	97.4
947697	EG10317	fis	Pseudomonas_aeruginosa	gi|9951124|gb|AAG08238.1|AE004898_9	61.97	8E-22	95.5
946423	EG10324	fliN	Clostridium_botulinum	CBO2743	49.41	6E-21	92.8
945069	EG11098	xseB	Haemophilus_influenzae	gi|1574276|gb|AAC23086.1|	61.33	1E-20	90.1
947286	EG10824	recB	Mycobacterium_tuberculosis_CDC1551	gi|16411737|emb|CAD00345.1|	22.62	1E-20	95.5
946005	G6751	b1440	Pseudomonas_aeruginosa	gi|9948656|gb|AAG05980.1|AE004688_2	25.07	4E-20	93.2
945069	EG11098	xseB	Vibrio_cholerae	gi|9655346|gb|AAF94053.1|	57.89	1E-19	87
946088	G6819	b1545	Clostridium_difficile	CD3578	33.16	2E-19	88.6
946088	G6819	b1545	Pseudomonas_aeruginosa	gi|9950046|gb|AAG07254.1|AE004804_2	35.85	2E-19	89.4
945120	G7433	ygcO	Salmonella_typhimurium_LT2	gi|16419871|gb|AAL20276.1|	47.95	3E-19	86.7
947286	EG10824	recB	Clostridium_difficile	CD0328	23.39	1E-17	86.3
948364	EG10209	dapF	Enterococcus_faecalis_V583	gi|29342554|gb|AAO80319.1|	28.46	3E-17	81.6
945861	G6816	ydfI	Clostridium_difficile	CD2331	24.29	5E-17	82.4
948589	G6653	ycjS	Clostridium_botulinum	CBO2004	27.08	9E-17	80.9
948589	G6653	ycjS	Bacillus_anthracis_Ames	gi|30257831|gb|AAP27060.1|	30.57	1E-16	80.5
948589	G6653	ycjS	Salmonella_typhimurium_LT2	gi|16419983|gb|AAL20384.1|	29.33	1E-16	80.9
946088	G6819	b1545	Clostridium_perfringens	gi|15076728|dbj|BAB62453.1|	26.49	1E-16	73.6
946716	EG10670	ompC	Vibrio_cholerae	gi|9655068|gb|AAF93799.1|	24.43	1E-16	80.5
948589	G6653	ycjS	Shigella_flexneri_2a	gi|24051978|gb|AAN43231.1|	27.65	3E-16	79.3
946005	G6751	b1440	Shigella_flexneri_2a	gi|56383294|gb|AAN42441.2|	26.78	3E-16	79.7
946005	G6751	b1440	Salmonella_typhimurium_LT2	gi|16419385|gb|AAL19813.1|	27.59	9E-16	78.2
945112	EG11703	acrA	Francisella_tularensis_tularensis	gi|56603772|emb|CAG44739.1|	23.62	1E-15	76.3
945861	G6816	ydfI	Enterococcus_faecalis_V583	gi|29342507|gb|AAO80272.1|	31.13	1E-15	77.4
946988	EG11072	xseA	Francisella_tularensis_tularensis	gi|56604747|emb|CAG45823.1|	22.3	1E-15	76.6
945112	EG11703	acrA	Vibrio_cholerae	gi|9655370|gb|AAF94075.1|	24.54	2E-15	76.3
947286	EG10824	recB	Staphylococcus_aureus_aureus_MRSA252	gi|49241256|emb|CAG39935.1|	21.04	9E-15	75.9
945069	EG11098	xseB	Pseudomonas_aeruginosa	gi|9950238|gb|AAG07429.1|AE004821_2	50	1E-14	72
946423	EG10324	fliN	Clostridium_difficile	CD0271	33.86	1E-14	72
948364	EG10209	dapF	Mycobacterium_tuberculosis_CDC1551	gi|16411471|emb|CAD00096.1|	28.21	2E-14	72.8
948589	G6653	ycjS	Enterococcus_faecalis_V583	gi|29343278|gb|AAO81041.1|	26.94	8E-14	70.9
946005	G6751	b1440	Mycobacterium_tuberculosis_CDC1551	gi|16410199|emb|CAC98888.1|	25.34	1E-13	70.5
946005	G6751	b1440	Yersinia_pestis_KIM	gi|21959748|gb|AAM86402.1|AE013887_9	25.76	2E-13	70.1
946088	G6819	b1545	Enterococcus_faecalis_V583	gi|29342616|gb|AAO80381.1|	29.12	2E-13	68.2
946005	G6751	b1440	Haemophilus_influenzae	gi|1574803|gb|AAC22988.1|	24.35	3E-13	68.6

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945861	G6816	ydfI	Streptococcus_pneumoniae_R6	gi|15457918|gb|AAK99163.1|	24.64	3E-13	68.9
946005	G6751	b1440	Staphylococcus_aureus_aureus_MRSA252	gi|49241394|emb|CAG40078.1|	26.32	4E-13	68.6
948589	G6653	ycjS	Pseudomonas_aeruginosa	gi|9951449|gb|AAG08534.1|AE004927_12	29.9	1E-12	68.2
945069	EG11098	xseB	Bacillus_anthracis_Ames	gi|30258899|gb|AAP28117.1|	42.25	2E-12	64.3
948589	G6653	ycjS	Streptococcus_pneumoniae_R6	gi|15459193|gb|AAL00334.1|	25.38	2E-12	65.5
945052	G7522	yqgC	Salmonella_typhimurium_LT2	gi|16421641|gb|AAL21963.1|	78.43	2E-12	64.3
948589	G6653	ycjS	Clostridium_difficile	CD2855	25.12	6E-12	65.1
946005	G6751	b1440	Bacillus_anthracis_Ames	gi|30255245|gb|AAP25254.1|	26.38	6E-12	65.5
945108	EG11704	acrB	Bacillus_anthracis_Ames	gi|30260133|gb|AAP29318.1|	21.9	1E-11	66.2
946005	G6751	b1440	Vibrio_cholerae	gi|9655923|gb|AAF94582.1|	25.6	1E-11	63.5
945069	EG11098	xseB	Enterococcus_faecalis_V583	gi|29343023|gb|AAO80786.1|	43.48	2E-11	60.1
946088	G6819	b1545	Clostridium_botulinum	CBO2046	34.95	2E-11	62
946005	G6751	b1440	Clostridium_botulinum	CBO0563	23.79	3E-11	62.8
946088	G6819	b1545	Streptococcus_pneumoniae_R6	gi|15458551|gb|AAK99746.1|	28.95	3E-11	60.5
947286	EG10824	recB	Clostridium_botulinum	CBO0683	19.4	3E-11	64.7
946005	G6751	b1440	Clostridium_difficile	CD1027	25.16	6E-11	62
946005	G6751	b1440	Enterococcus_faecalis_V583	gi|29344599|gb|AAO82357.1|	23.97	7E-11	61.2
945108	EG11704	acrB	Staphylococcus_aureus_aureus_MRSA252	gi|49242605|emb|CAG41326.1|	24.16	1E-10	62
945108	EG11704	acrB	Clostridium_difficile	CD2407	25	2E-10	62
945069	EG11098	xseB	Mycobacterium_tuberculosis_CDC1551	gi|16410778|emb|CAC99440.1|	39.44	8E-10	54.7
946005	G6751	b1440	Streptococcus_pneumoniae_R6	gi|15458881|gb|AAL00047.1|	21.97	2E-09	56.2
949035	EG11002	thyA	Clostridium_botulinum	CBO0768	21.31	2E-09	56.2
947362	EG11071	xerD	Streptococcus_pneumoniae_R6	gi|15458015|gb|AAK99251.1|	30	2E-09	55.5
945861	G6816	ydfI	Staphylococcus_aureus_aureus_MRSA252	gi|49242510|emb|CAG41228.1|	28.31	4E-09	55.8
945484	EG10852	rimK	Francisella_tularensis_tularensis	gi|56603836|emb|CAG44811.1|	20.98	5E-09	53.9
945108	EG11704	acrB	Francisella_tularensis_tularensis	gi|56603771|emb|CAG44738.1|	28.21	9E-09	55.1
946088	G6819	b1545	Mycobacterium_tuberculosis_CDC1551	gi|16411821|emb|CAD00410.1|	24.74	1E-08	52.8
947053	EG12341	rseA	Pseudomonas_aeruginosa	gi|9946651|gb|AAG04152.1|AE004511_5	32.61	2E-08	53.1
945069	EG11098	xseB	Streptococcus_pneumoniae_R6	gi|15458711|gb|AAK99891.1|	39.34	3E-08	48.9
946423	EG10324	fliN	Bacillus_anthracis_Ames	gi|30255996|gb|AAP25631.1|	26.74	3E-08	50.8
945108	EG11704	acrB	Vibrio_cholerae	gi|9654566|gb|AAF93340.1|	21.92	5E-08	53.5
945069	EG11098	xseB	Clostridium_botulinum	CBO1884	34.78	8E-08	48.1
946423	EG10324	fliN	Mycobacterium_tuberculosis_CDC1551	gi|16410089|emb|CAC98778.1|	30.77	1E-07	48.1
947521	EG11009	tolC	Staphylococcus_aureus_aureus_MRSA252	gi|49241754|emb|CAG40444.1|	20.56	2E-07	50.4
945069	EG11098	xseB	Staphylococcus_aureus_aureus_MRSA252	gi|49241901|emb|CAG40595.1|	35	4E-07	45.4
945112	EG11703	acrA	Haemophilus_influenzae	gi|1573913|gb|AAC22554.1|	24.05	4E-07	48.1

Figure 12 (page 11 of 11)

Escherichia coli K-12 RecA Protein

GenBank accession number AAC75741.1, GeneID 947170

MAIDENKQKALAAALGQIEKQFGKGSIMRLGEDRSMDVETISTGSLSLDIALGAGGLPMGRIVE
IYGPESSGKTTLTLQVIAAAQREGKTCAFIDAEHALDPIYARKLGVDIDNLLCSQPDTGEQALE
ICDALARSGAVDVIVVDSVAALTPKAEIEGEIGDSHMGLAARMMSQAMRKLAGNLKQSNTLLIF
INQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRIGAVKEGENVVGSETRVKVVKNKIAAPF
KQAEFQILYGEGINFYGELVDLGVKEKLIEKAGAWYSYKGEKIGQGKANATAWLKDNPETAKEI
EKKVRELLLSNPNSTPDFSVDDSEGVAETNEDF (SEQ ID NO: 1)

Staphylococcus aureus aureus MRSA252 Rec A Protein

UniProt accession number CAG40263.1

MDNDRQKALDTVIKNMEKSFGKGAVMKLGDNIGRRVSTTSTGSVTLDNALGVGGYPKGRIIEIY
GPESSGKTTVALHAIAEVQSNGGVAAFIDAEHALDPEYAQALGVDIDNLYLSQPDHGEQGLEIA
EAFVRSGAVDIVVVDSVAALTPKAEIEGEMGDTHVGLQARLMSQALRKLSGAISKSNTTAIFIN
QIREKVGVMFGNPETTPGGRALKFYSSVRLEVRRAEQLKQGQEIVGNRTKIKVVKNKVAPPFRV
AEVDIMYGQGISKEGELIDLGVENDIVDKSGAWYSYNGERMGQGKENVKMYLKENPQIKEEIDR
KLREKLGISDGDVEETEDAPKSLFDEE (SEQ ID NO: 2)

Pseudomonas aeruginosa RecA Protein

GenBank accession number AE004782_3

MDENKKRALAAALGQIERQFGKGAVMRMGDHERQAIPAISTGSLGLDIALGIGGLPKGRIVEIY
GPESSGKTTLTLSVIAEAQKQGATCAFVDAEHALDPDYAGKLGVNVDDLLVSQPDTGEQALEIT
DMLVRSNAVDVIIVDSVAALVPKAEIEGEMGDAHVGLQARLMSQALRKITGNIKNANCLVIFIN
QIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRTGAVKEGDEVVGSETRVKVVKNKVSPPFRQ
AEFQILYGKGIYRTGEIIDLGVQLGLVEKSGAWYSYQGSKIGQGKANAAKYLEDNPEIGSVLEK
TIRDQLLAKSGPVKADAEEVADAEAD (SEQ ID NO: 3)

Figure 13

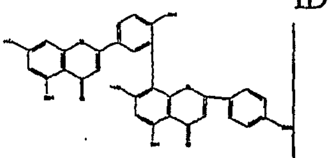
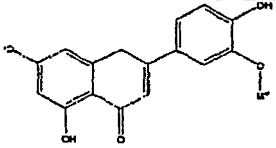
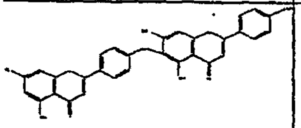
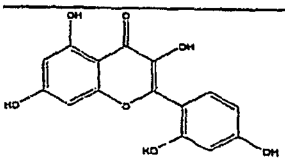
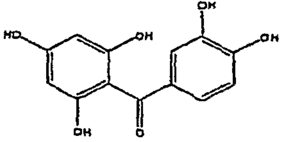
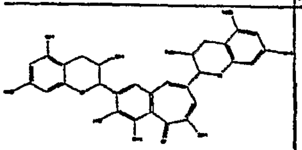
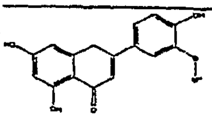
Structure	Database ID	Chemical Name	Percent Inhibition	µg/mL	IC$_{50}$ (µg/mL)
	IF_02-B09	Amentoflavone	109	100	5
	IF_01-A06	ISORHAMNETIN	100	100	
	IF_01-G05	HINOKIFLAVONE	90	100	1
	IF_02-A10	MORIN	88	100	10
	IF_02-B08	Maclurin	86	100	15
	IF_02-E07	theaflavin	86	50	1.5
	IF_02-A06	KAEMPFEROL	70	100	5

Figure 14 (page 1 of 2)

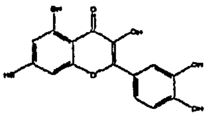
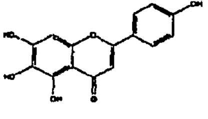
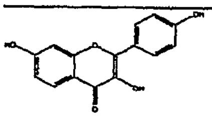
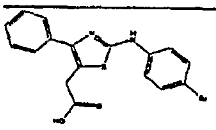
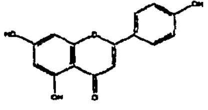
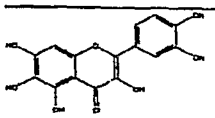
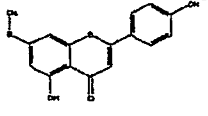
Structure	Database ID	Chemical Name	Percent Inhibition	µg/mL	IC$_{50}$ (µg/mL)
	CD_01-G08	quercetin dihydrate	67	200	-1
	IF_02-A08	6-HYDROXYAPIGENIN	65	100	3
	IF_02-A05	3,7,4'-TRIHYDROXYFLAVONE	63	100	10
	CD_01-C03		61	200	100
	IF_02-E03	apigenin	56	50	3
	IF_02-A03	QUERCETAGETIN	55	100	1.6
	IF_01-A02	APIGENIN-7-O-GLUCOSIDE	48	100	-1

Figure 14 (page 2 of 2)

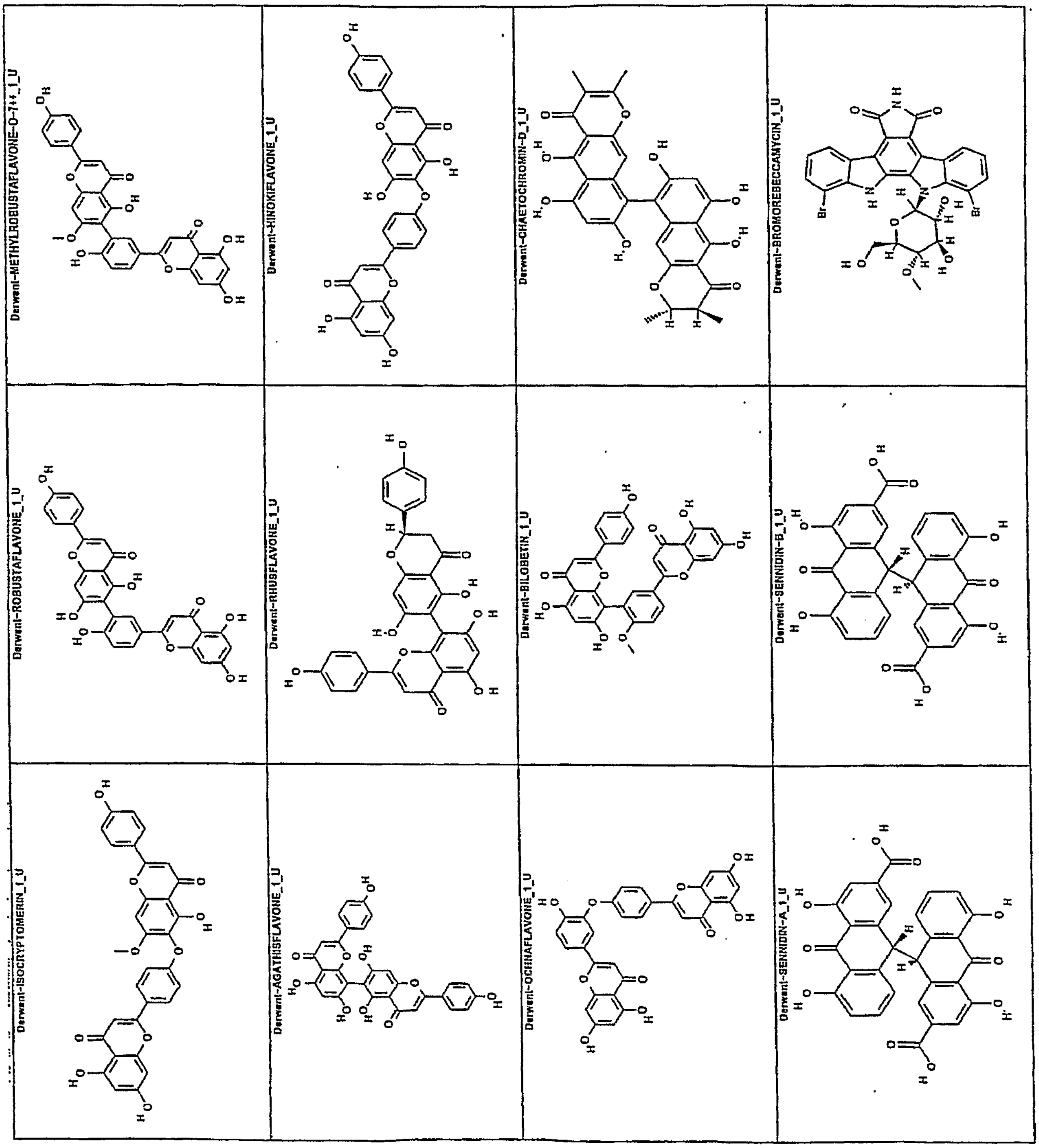

Derwent-ISOCRYPTOMERIN_1_U
Derwent-ROBUSTAFLAVONE_1_U
Derwent-METHYLROBUSTAFLAVONE-O-7‡‡_1_U
Derwent-AGATHISFLAVONE_1_U
Derwent-RHUSFLAVONE_1_U
Derwent-HINOKIFLAVONE_1_U
Derwent-OCHNAFLAVONE_1_U
Derwent-BILOBETIN_1_U
Derwent-CHAETOCHROMIN-D_1_U
Derwent-SENNIDIN-A_1_U
Derwent-SENNIDIN-B_1_U
Derwent-BROMOREBECCAMYCIN_1_U

Figure 15

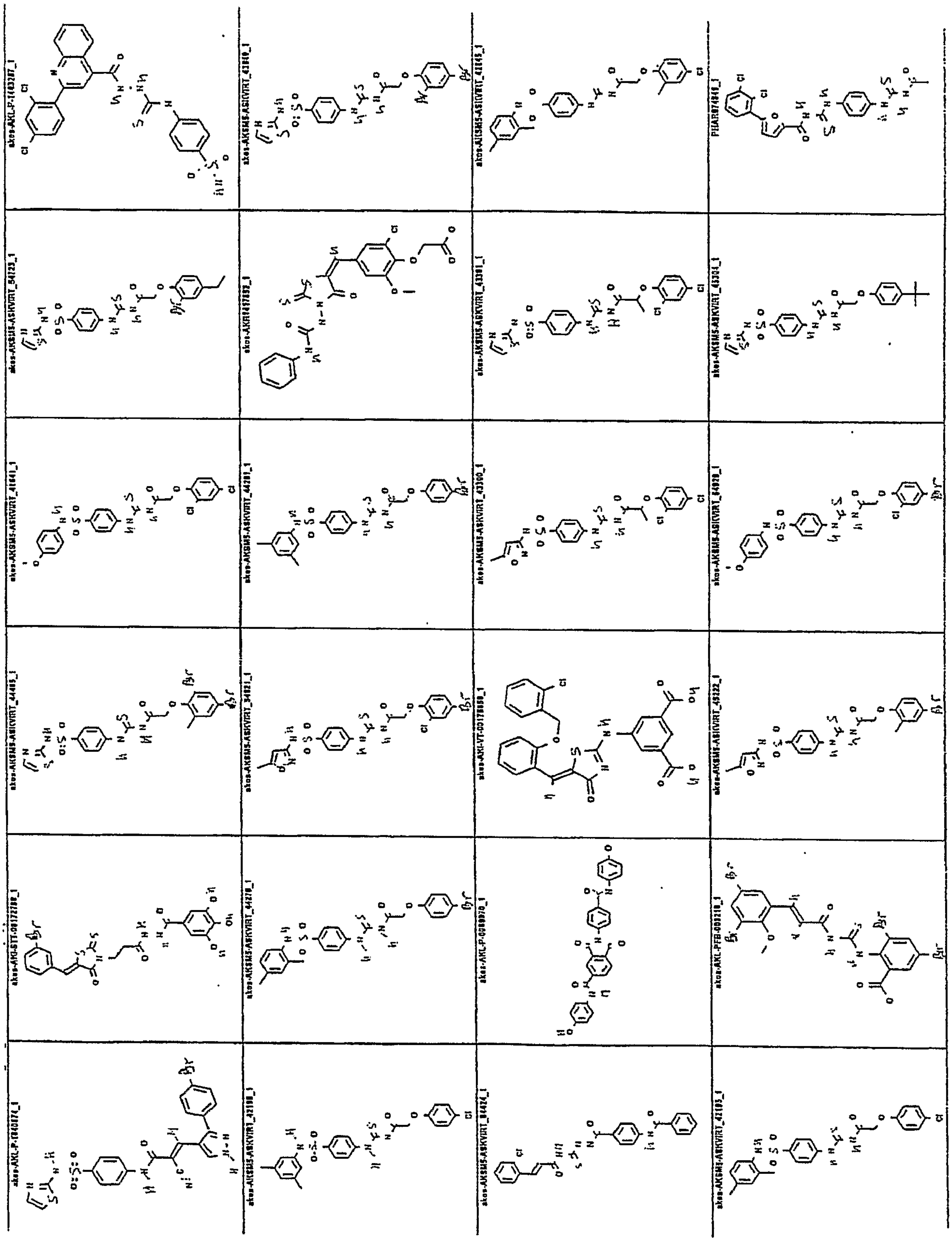


Figure 16 (page 1 of 2)

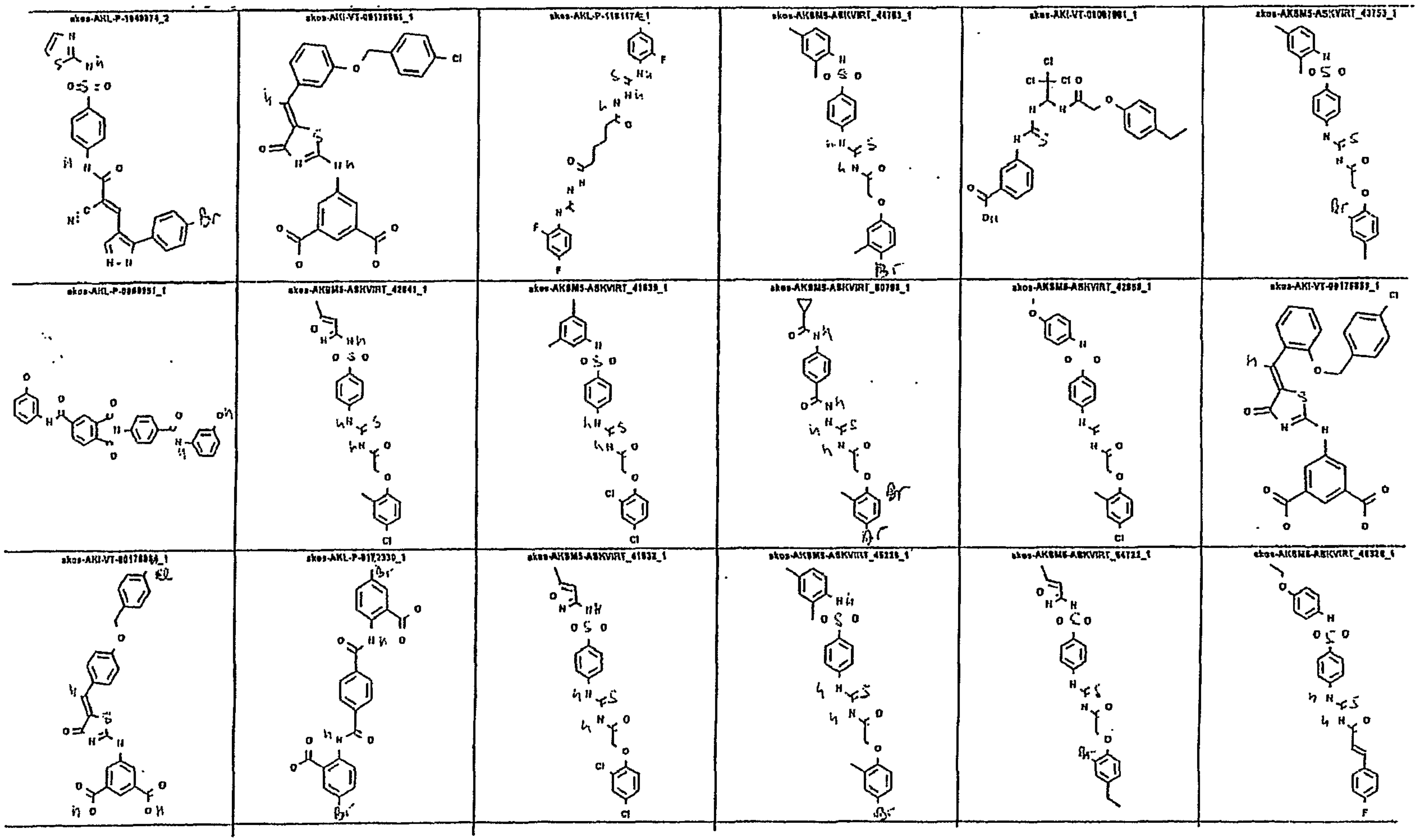


Figure 16 (page 2 of 2)

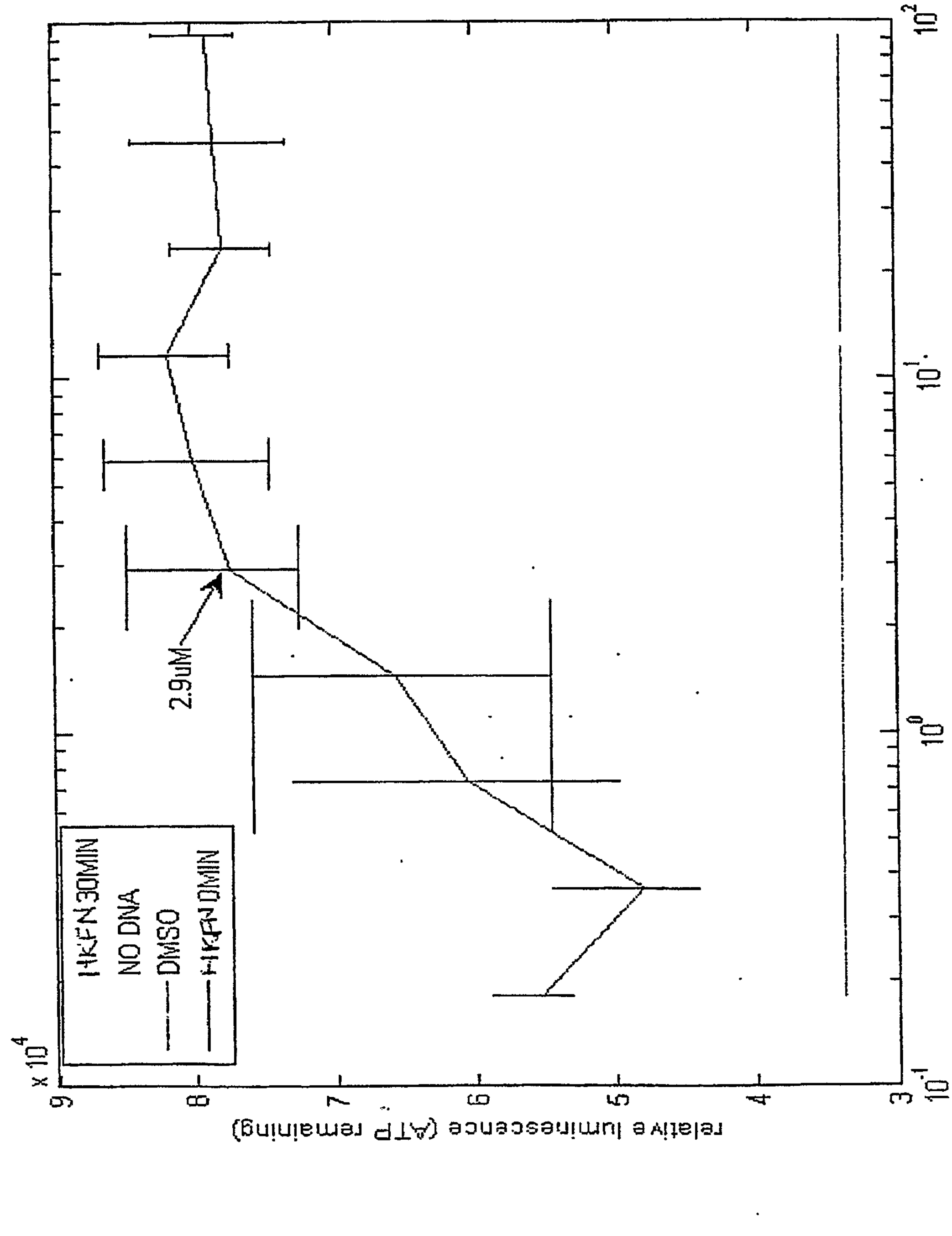

x 10^4
9
8
7
6
5
4
3
relative luminescence (ATP remaining)
HKFN 30MIN
NO DNA
DMSO
HKFN 0MIN
2.9uM
10^-1
10^0
10^1
10^2
HINOKIFLAVONE (uM)

Figure 17

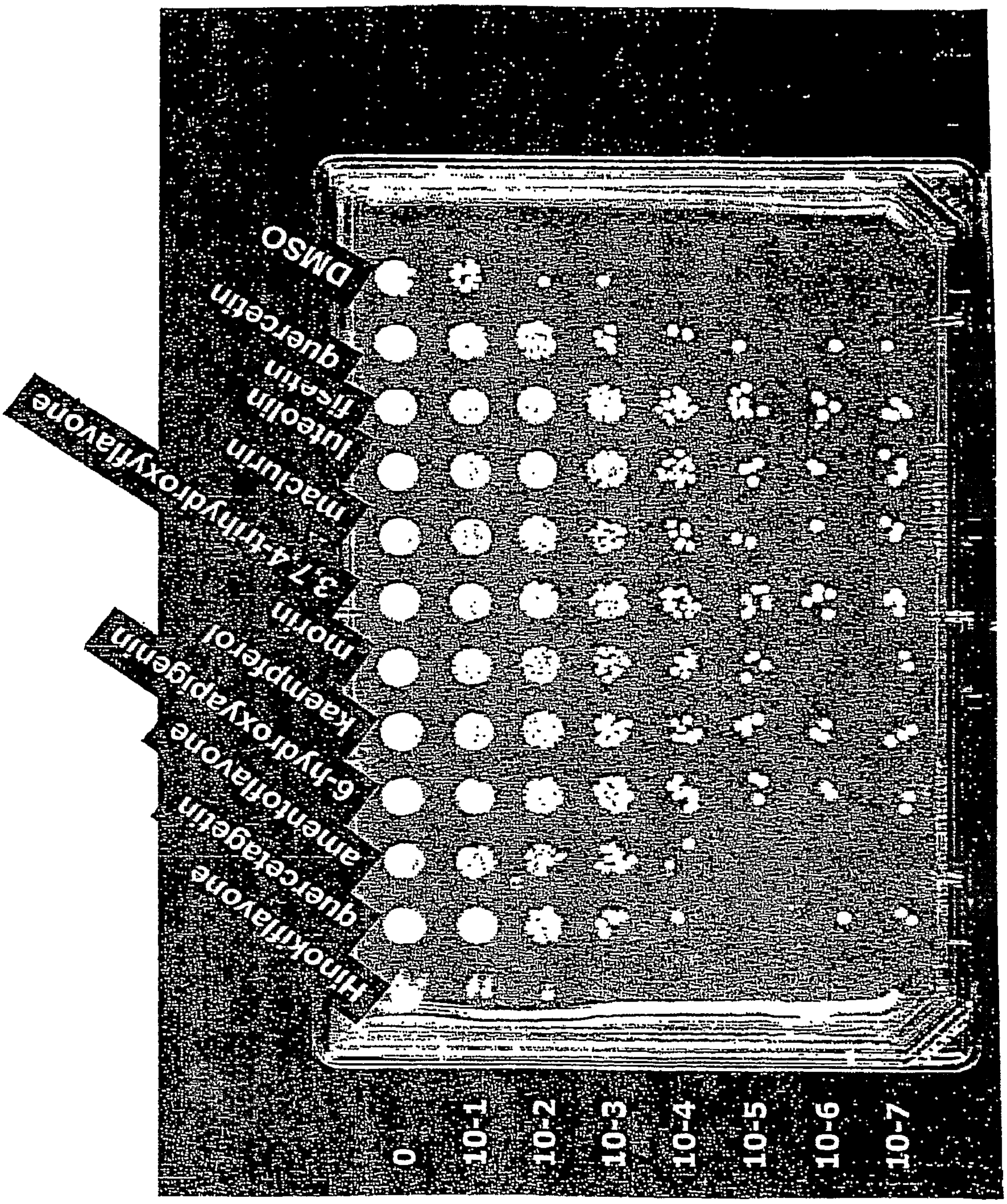

DMSO
quercetin
fisetin
luteolin
maclurin
3,7,4-trihydroxyflavone
morin
kaempferol
6-hydroxyapigenin
amentoflavone
quercetagetin
Hinokiflavone
0
10-1
10-2
10-3
10-4
10-5
10-6
10-7

Figure 18

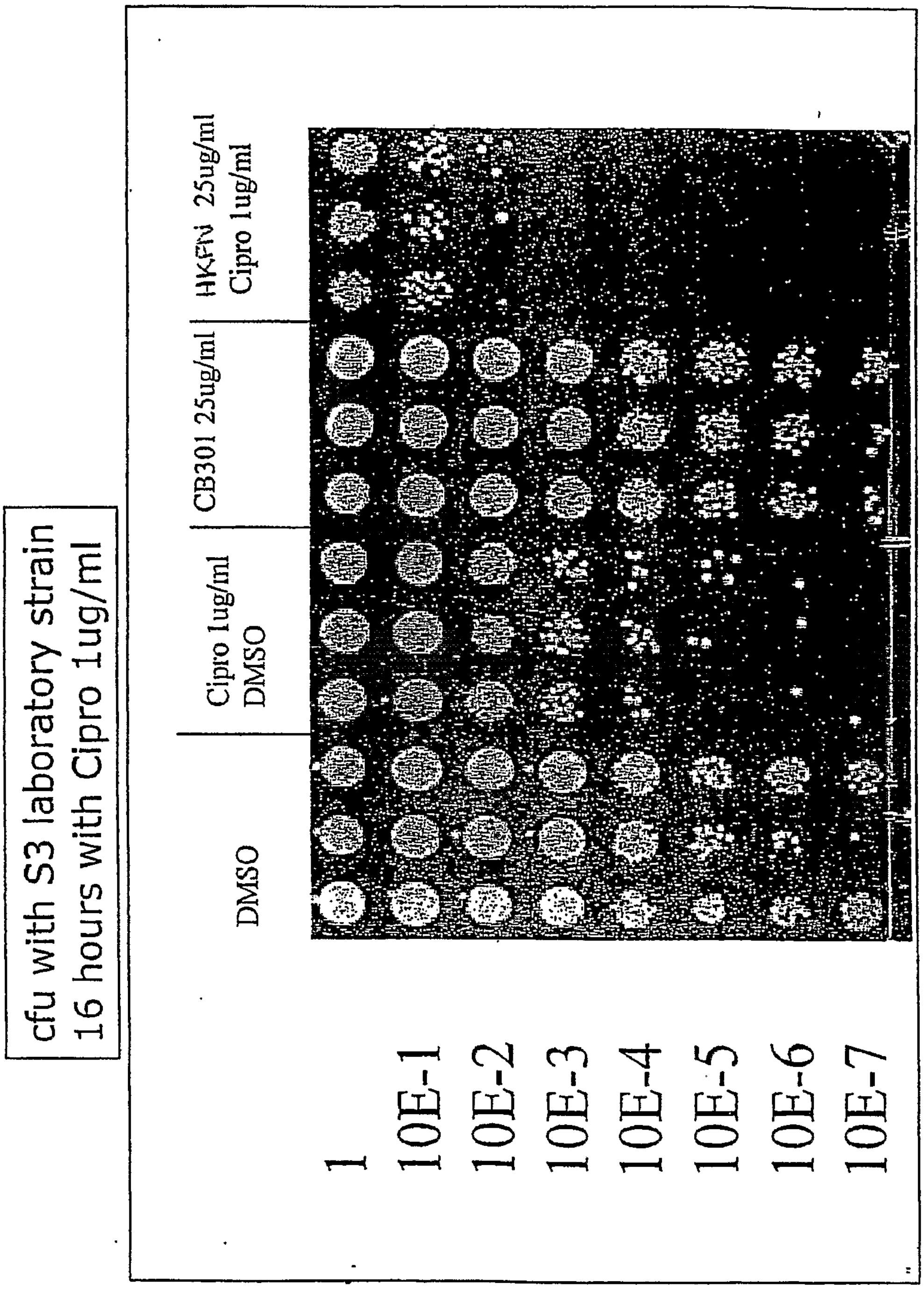

cfu with S3 laboratory strain
16 hours with Cipro 1ug/ml
DMSO
Cipro 1ug/ml
DMSO
CB301 25ug/ml
HKFN 25ug/ml
Cipro 1ug/ml
1
10E-1
10E-2
10E-3
10E-4
10E-5
10E-6
10E-7

Figure 19

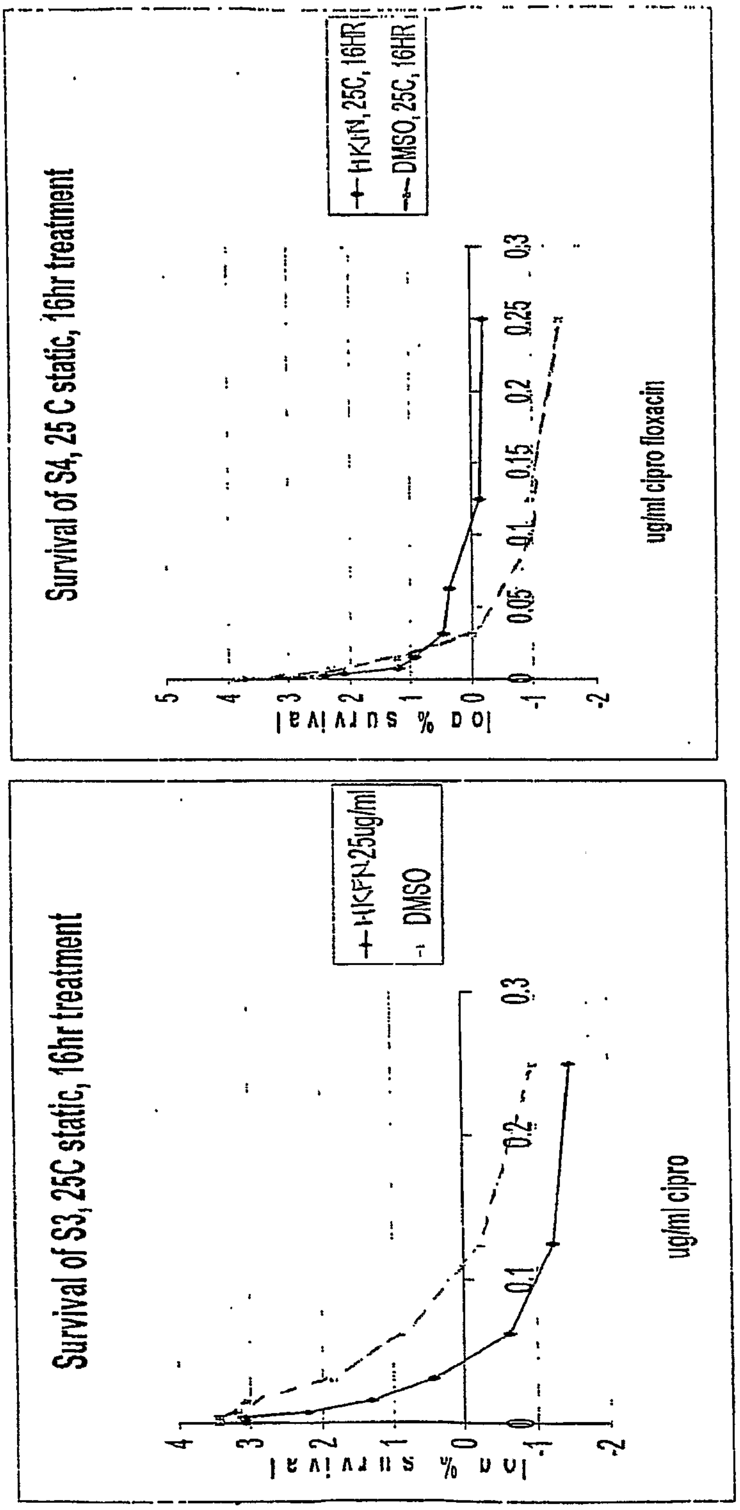

Survival of S3, 25C static, 16hr treatment
HKFN 25ug/ml
DMSO
log % survival
4
3
2
1
0
-1
-2
0
0.1
0.2
0.3
ug/ml cipro
Survival of S4, 25 C static, 16hr treatment
HKFN, 25C, 16HR
DMSO, 25C, 16HR
log % survival
5
4
3
2
1
0
-1
-2
0
0.05
0.1
0.15
0.2
0.25
0.3
ug/ml cipro floxacin

Figure 20

Prediction Method and Predicted Binding Sites

- X-ray structure based (1xmv)
- All waters and ligands removed
- Whole protein surface covered

- Predicts druggable sites for small molecules

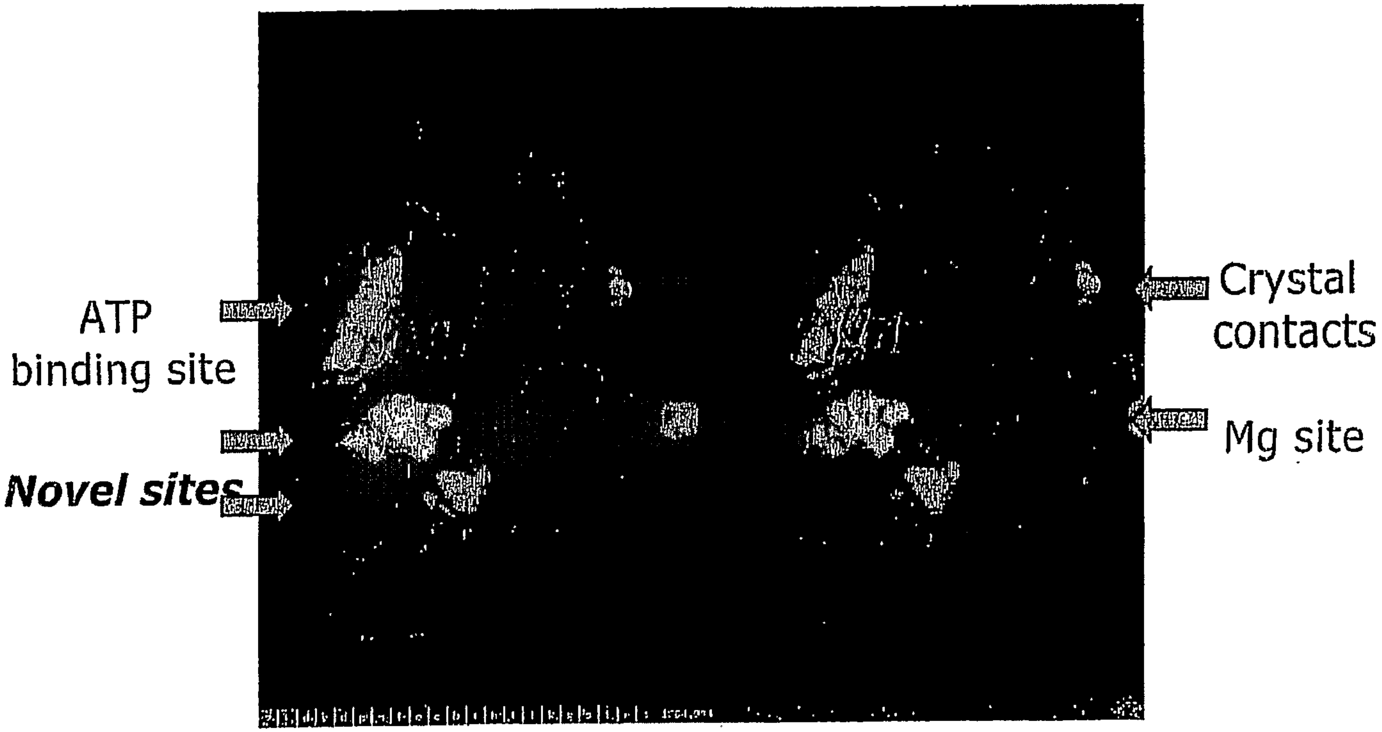
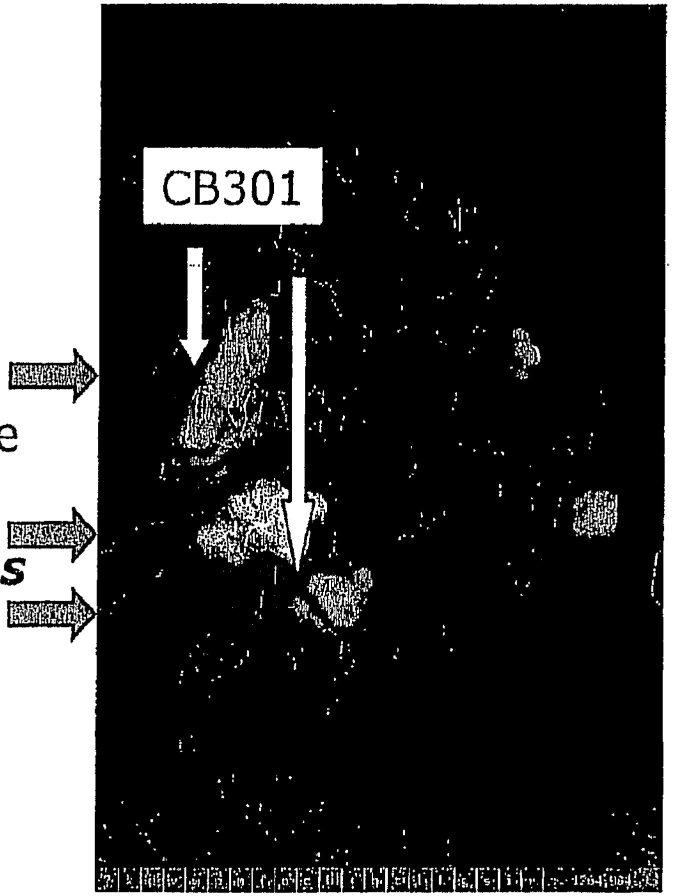


Figure 21

Predicted Binding Sites, Predicted Inhibitory Sites



ATP binding site

Novel sites

Figure 22

Prediction results

- Known ADP/ATP site correctly predicted
- Known additional functional sites from crystal contacts or ion binding sites correctly predicted
- 2 novel sites, one as populated as ADP/ATP
- Characterisation of ATP-analogs (JMedChem) did reveal complex inhibition mechanism, second site reasonable

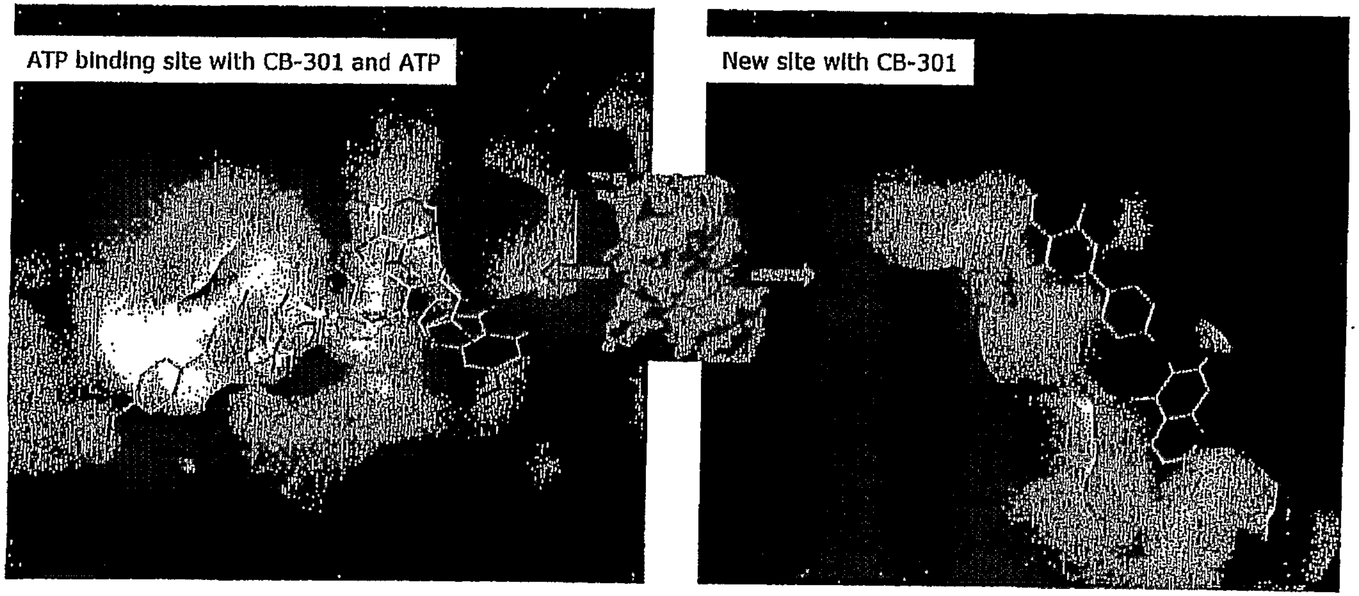


Figure 23

Novel binding site situated around

R324

R85 *F270* *Y271*

K310

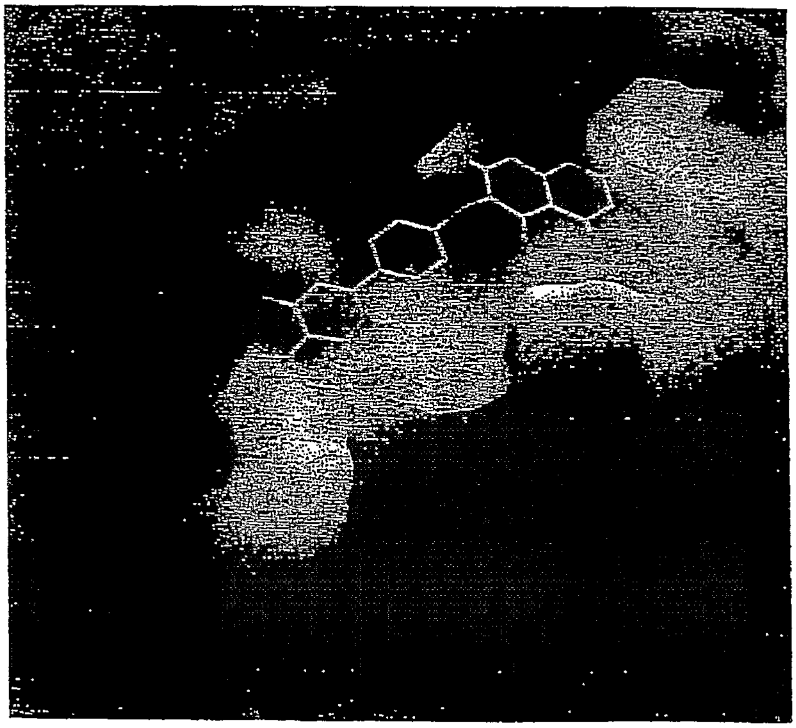
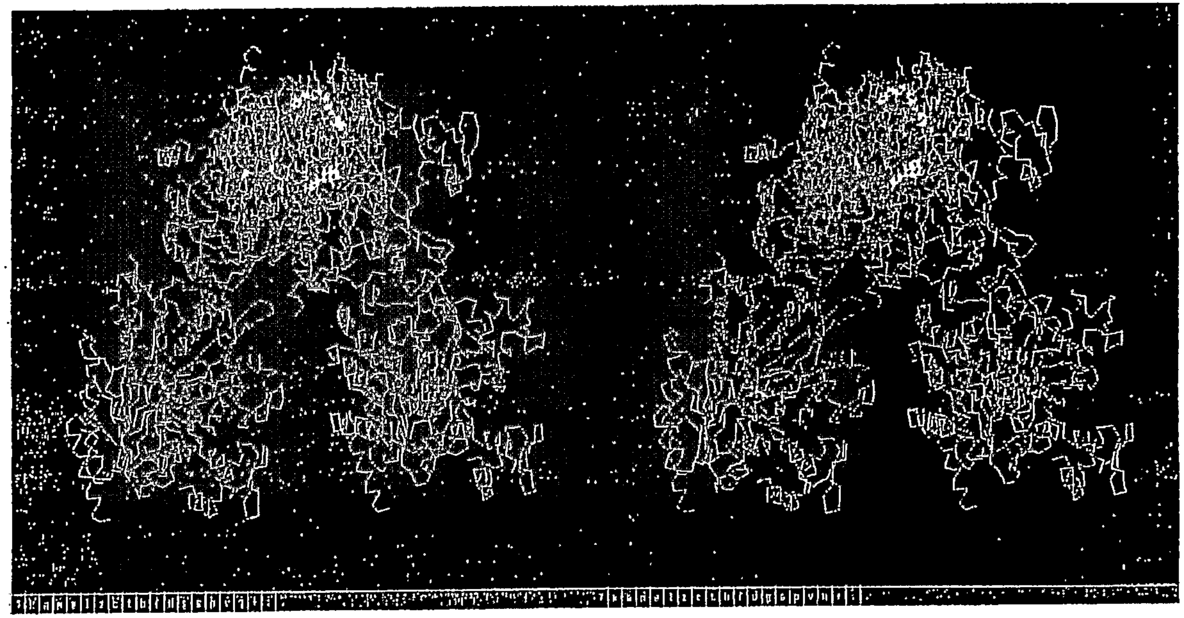


Figure 24

Position in Filament



While the ATP site is situated in the contact region of recA domains,

The novel site is directed outwards

Figure 25

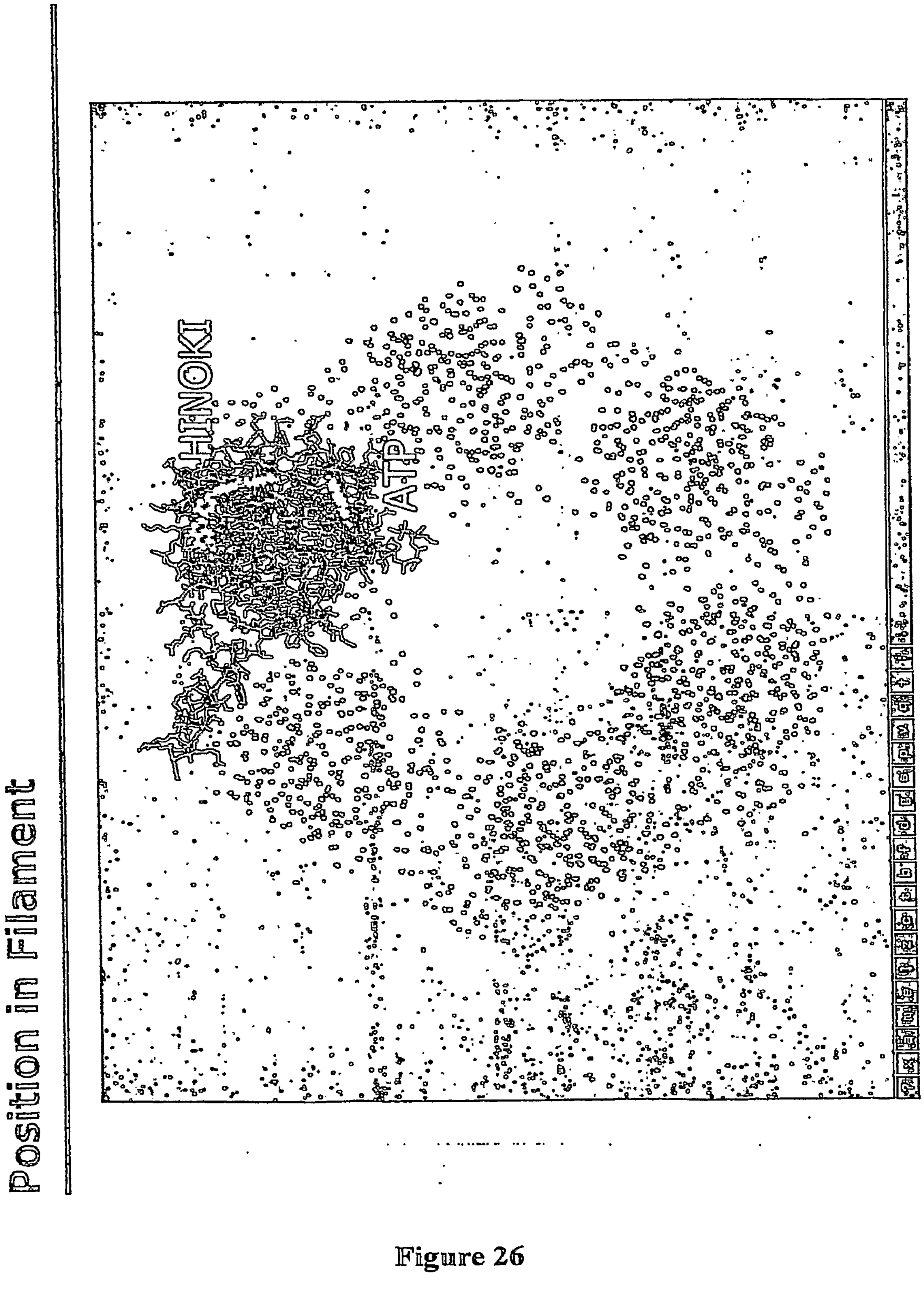


Figure 26

Hinokiflavone energy mapping

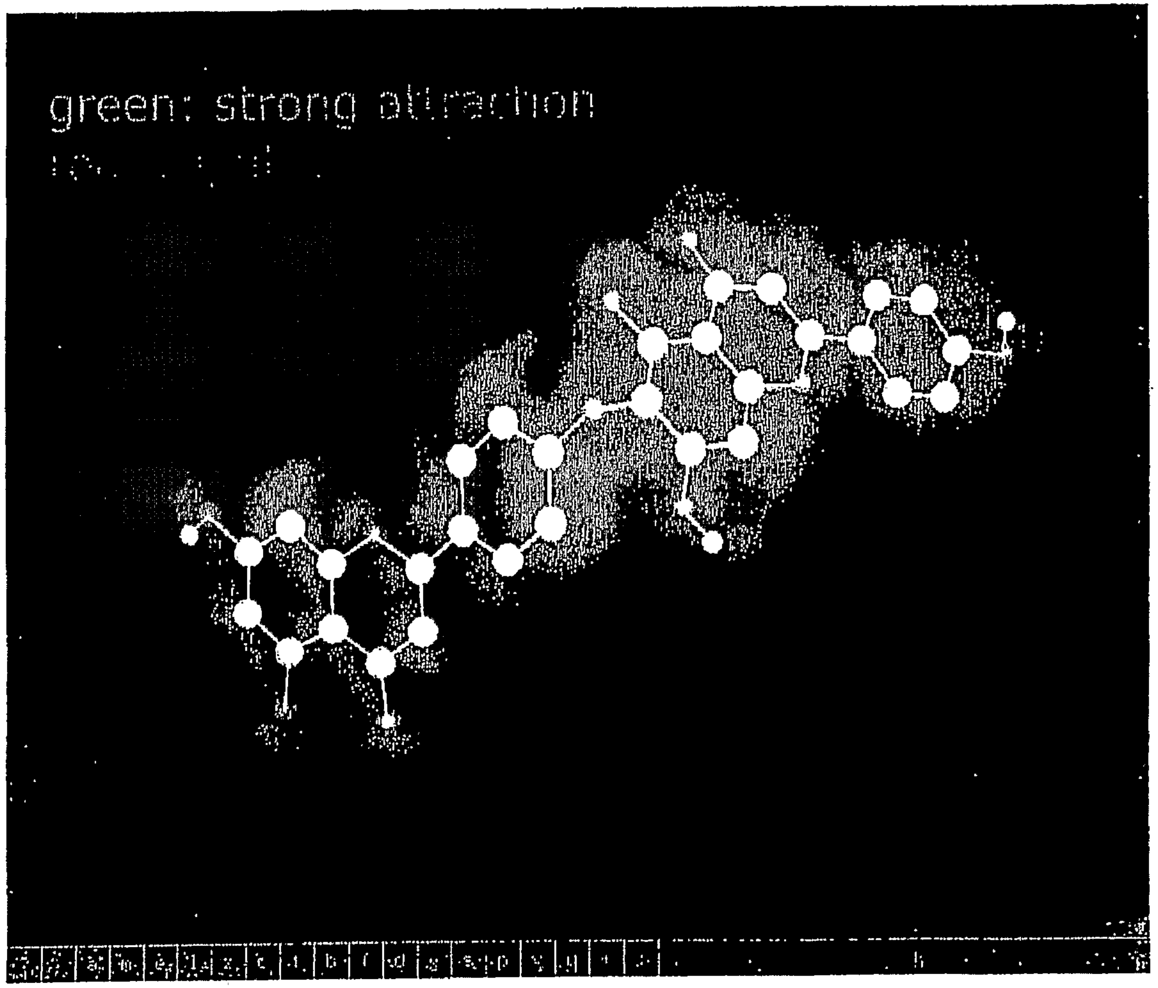


Figure 27

New Site

1xmv	1g18	1ubc	1xp8
site detected	site detected	site detected	site detected
E. coli	M. tuberculosis	M. smegmatis	D. radiodurans
Gram-	Gram+	Gram+	Gram+

The novel site is found in all species investigated
– Gram positive and negative –

With little changes in sequence

⇨ **Broad spectrum recA inhibitors possible**

Figure 28

Gene	BLAT_ID	Description
aceE	b0114	pyruvate dehydrogenase (decarboxylase component)
araJ	b0396	involved in either transport or processing of arabinose polymers
aspA	b4139	aspartate ammonia-lyase (aspartase)
atpA	b3734	membrane-bound ATP synthase, F1 sector, alpha-subunit
atpF	b3736	membrane-bound ATP synthase, F0 sector, subunit b
atpH	b3735	membrane-bound ATP synthase, F1 sector, delta-subunit
brnQ	b0401	branched chain amino acid transport system II carrier protein
cydB	b0734	cytochrome d terminal oxidase polypeptide subunit II
degP		
dnaT	b4362	DNA biosynthesis; primosomal protein i
fepD	b0590	ferric enterobactin (enterochelin) transport
folP	b3177	7,8-dihydropteroate synthase
guaB	b2508	IMP dehydrogenase
hfq	b4172	host factor I for bacteriophage Q beta replication, a growth-related protein
iscS	b2530	putative aminotransferase
JW5360		
lipA	b0628	lipoate synthesis, sulfur insertion
lpdA	b0116	lipoamide dehydrogenase (NADH); component of 2-oxodehydrogenase and pyruvate complexes; L-protein of glycine cleavage complex
nuoB	b2287	NADH dehydrogenase I chain B
nuoJ	b2280	NADH dehydrogenase I chain J
nuoL	b2278	NADH dehydrogenase I chain L
osmB	b1283	osmotically inducible lipoprotein
pdxH	b1638	pyridoxinephosphate oxidase
pnuC	b0751	required for NMN transport
priA	b3935	primosomal protein N'(= factor Y)(putative helicase)
priB	b4201	primosomal replication protein N
rbfA	b3167	ribosome-binding factor A
recA	b2699	DNA strand exchange and renaturation, DNA-dependent ATPase, DNA- and ATP-dependent coprotease
rfaD	b3619	ADP-L-glycero-D-mannoheptose-6-epimerase
rpmJ	b3299	50S ribosomal subunit protein L36
rpoD	b3067	RNA polymerase, sigma(70) factor; regulation of proteins induced at high temperatures
rseA	b2572	sigma-E factor, negative regulatory protein
ruvC	b1863	Holliday junction nuclease; resolution of structures; repair
sdhC	b0721	succinate dehydrogenase, cytochrome b556
seqA	b0687	negative modulator of initiation of replication
setA		
sucB	b0727	2-oxoglutarate dehydrogenase (dihydrolipoyltranssuccinase E2 component)
surA	b0053	survival protein
tar	b1886	methyl-accepting chemotaxis protein II, aspartate sensor receptor
tatE		
tauB	b0366	taurine ATP-binding component of a transport system
trmU		
ubiH	b2907	2-octaprenyl-6-methoxyphenol--> 2-octaprenyl-6-methoxy-1, 4-benzoquinone
ubiX	b2311	3-octaprenyl-4-hydroxybenzoate carboxy-lyase
xerC	b3811	site-specific recombinase, acts on cer sequence of ColE1, effects chromosome segregation at cell division
yaaU	b0045	putative transport protein
ycfM	b1105	orf, hypothetical protein
yejO	b2190	putative ATP-binding component of a transport system
ygfZ	b2898	orf, hypothetical protein
ygiH	b3059	orf, hypothetical protein
yheL	b3343	orf, hypothetical protein
yheM	b3344	orf, hypothetical protein
yheN	b3345	orf, hypothetical protein
yicG	b3646	orf, hypothetical protein
yjdL	b4130	putative peptide transporter
yjeT	b4176	orf, hypothetical protein
yjiO	b4337	putative transport protein
yjjY	b4402	orf, hypothetical protein

Figure 29

S. aureus recA- is more sensitive to aminoglycosides

cfu S3 (parent) and S4 (recA defective),
Survival after 4 hours with Gentamicin

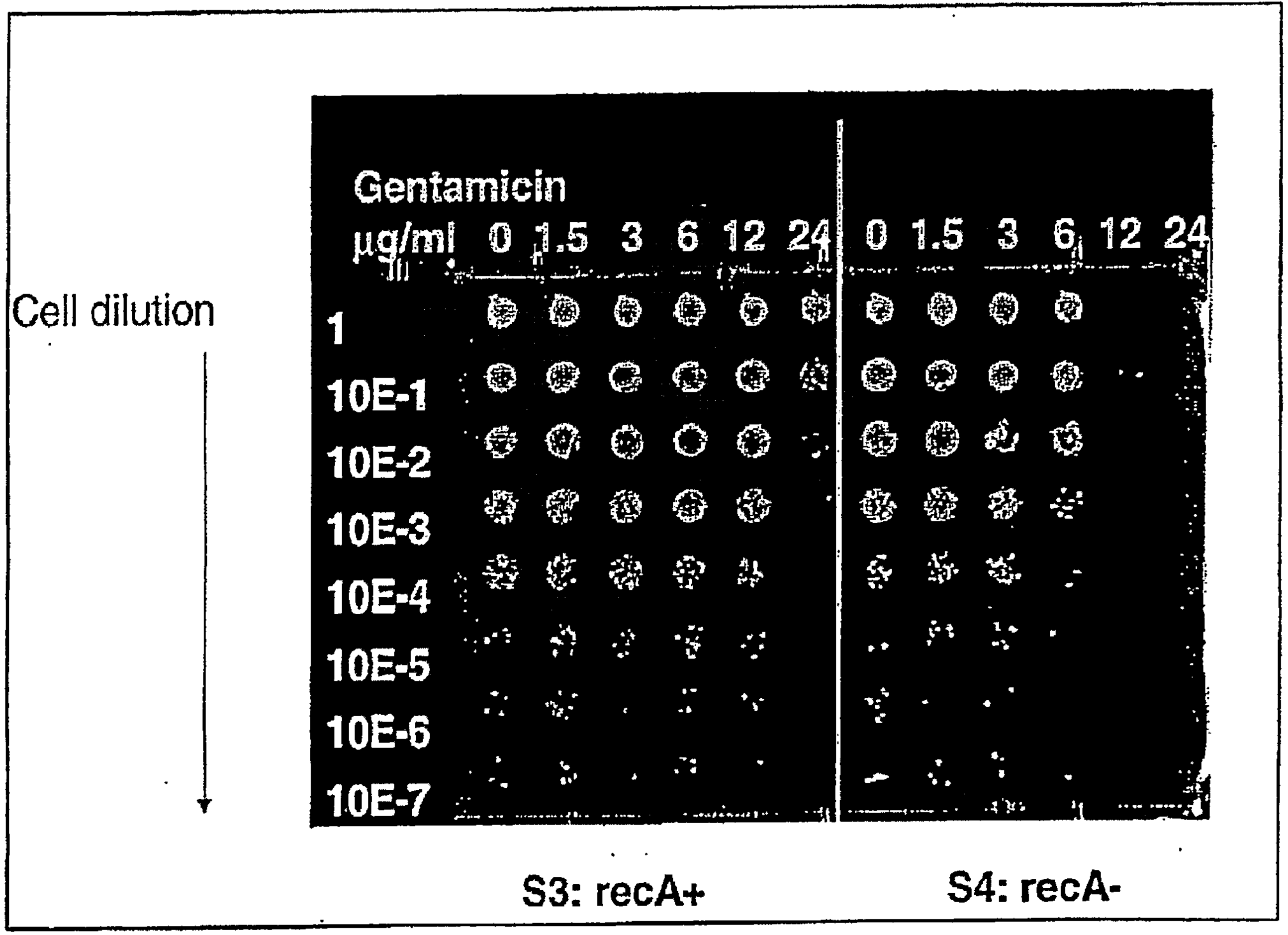
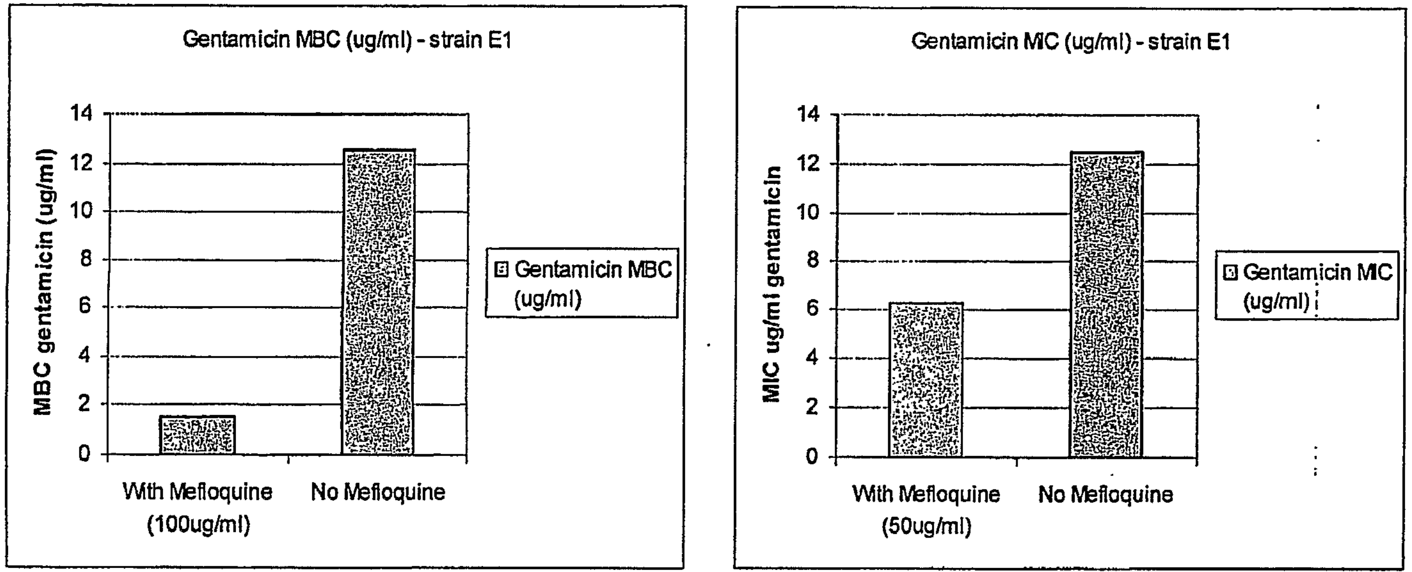


Figure 30

Mefloquine potentiate Gentamicin



Mefloquine reduced the MBC of Gentamicin by 6 fold

Mefloquine reduced the MIC of Gentamicin by 2 fold

Figure 31

Gene	BLAT_ID	Description
agaA	b3135	putative N-acetylgalactosamine-6-phosphate deacetylase
atpA	b3734	membrane-bound ATP synthase, F1 sector, alpha-subunit
atpF	b3736	membrane-bound ATP synthase, F0 sector, subunit b
atpH	b3735	membrane-bound ATP synthase, F1 sector, delta-subunit
bglF	b3722	PTS system beta-glucosides, enzyme II, cryptic
cysE	b3607	serine acetyltransferase
cysI	b2763	sulfite reductase, alpha subunit
fepC	b0588	ATP-binding component of ferric enterobactin transport
fepD	b0590	ferric enterobactin (enterochelin) transport
frvR	b3897	putative frv operon regulatory protein
guaA	b2507	GMP synthetase (glutamine-hydrolyzing)
guaB	b2508	IMP dehydrogenase
hit >1		
hofF	b3327	putative general protein secretion protein
hsdS	b4348	specificity determinant for hsdM and hsdR
iscS	b2530	putative aminotransferase
JW4016		
JW5075		
JW5227		
JW5257		
JW5360		
kdgK	b3526	ketodeoxygluconokinase
lipA	b0628	lipoate synthesis, sulfur insertion?
lysA	b2838	diaminopimelate decarboxylase
malG	b4032	part of maltose permease, inner membrane
mbhA	b0230	putative motility protein
mdoG	b1048	periplasmic glucans biosynthesis protein
nei	b0714	endonuclease VIII and DNA N-glycosylase with an AP lyase activity
nmpC	b0553	outer membrane porin protein; locus of qsr prophage
nudH		
pdxH	b1638	pyridoxinephosphate oxidase
phnB	b4107	orf, hypothetical protein
phnL	b4096	ATP-binding component of phosphonate transport
phnO	b4093	putative regulator, phn operon
pnuC	b0751	required for NMN transport
potE	b0692	putrescine transport protein
pshM	b3334	putative general secretion
ptsA	b3947	PEP-protein phosphotransferase system enzyme I

Figure 32 (page 1 of 2)

Gene	BLAT_ID	Description
rhaT	b3907	rhamnose transport
rpiA	b2914	ribosephosphate isomerase, constitutive
rseA	b2572	sigma-E factor, negative regulatory protein
sbp	b3917	periplasmic sulfate-binding protein
speA	b2938	biosynthetic arginine decarboxylase
sucB	b0727	2-oxoglutarate dehydrogenase (dihydrolipoyltranssuccinase E2 component)
sugE	b4148	suppresses groEL, may be chaperone
tdcE	b3114	probable formate acetyltransferase 3
tdcG	b3035	outer membrane channel; specific tolerance to colicin E1; segregation of daughter chromosomes
tolC	b3781	thioredoxin 1
trxA	b3833	2-octaprenyl-6-methoxy-1,4-benzoquinone --> 2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone
ubiE	b2907	2-octaprenyl-6-methoxyphenol--> 2-octaprenyl-6-methoxy-1, 4-benzoquinone
ubiH	b2311	3-octaprenyl-4-hydroxybenzoate carboxy-lyase
ubiX	b3035	outer membrane channel; specific tolerance to colicin E1; segregation of daughter chromosomes
xni	b0513	putative transport
ybbY	b1105	orf, hypothetical protein
ycfM	b1537	orf, hypothetical protein
ydeJ	b2015	putative transcriptional regulator LYSR-type
yeeY	b2427	orf, hypothetical protein
yfeT	b2709	putative 2-component transcriptional regulator
ygaA	b0513	putative transport
ygfZ	b2898	orf, hypothetical protein
yhdX	b3269	putative transport system permease protein
yheL	b3343	orf, hypothetical protein
yheM	b3344	orf, hypothetical protein
yiaY	b3589	putative oxidoreductase
yidK	b3679	putative cotransporter
yihV	b3883	putative kinase
yjbN	b4049	orf, hypothetical protein
yjcR	b4082	putative membrane protein
yjcZ	b4110	orf, hypothetical protein
ynjD		
yqeC		
yqiH	b3047	putative membrane protein
yrfA	b3392	orf, hypothetical protein

Figure 32 (page 2 of 2)

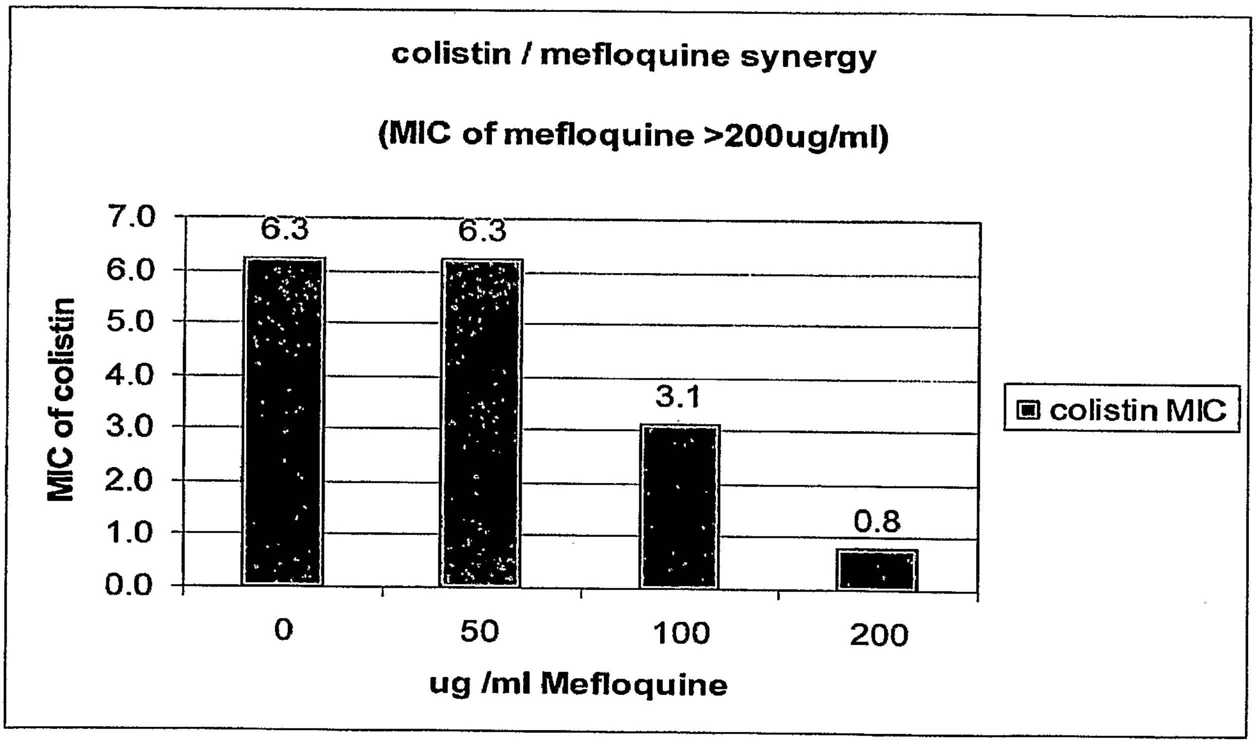

colistin / mefloquine synergy
(MIC of mefloquine >200ug/ml)
MIC of colistin
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
6.3
6.3
3.1
0.8
0
50
100
200
ug /ml Mefloquine
■ colistin MIC

Figure 33

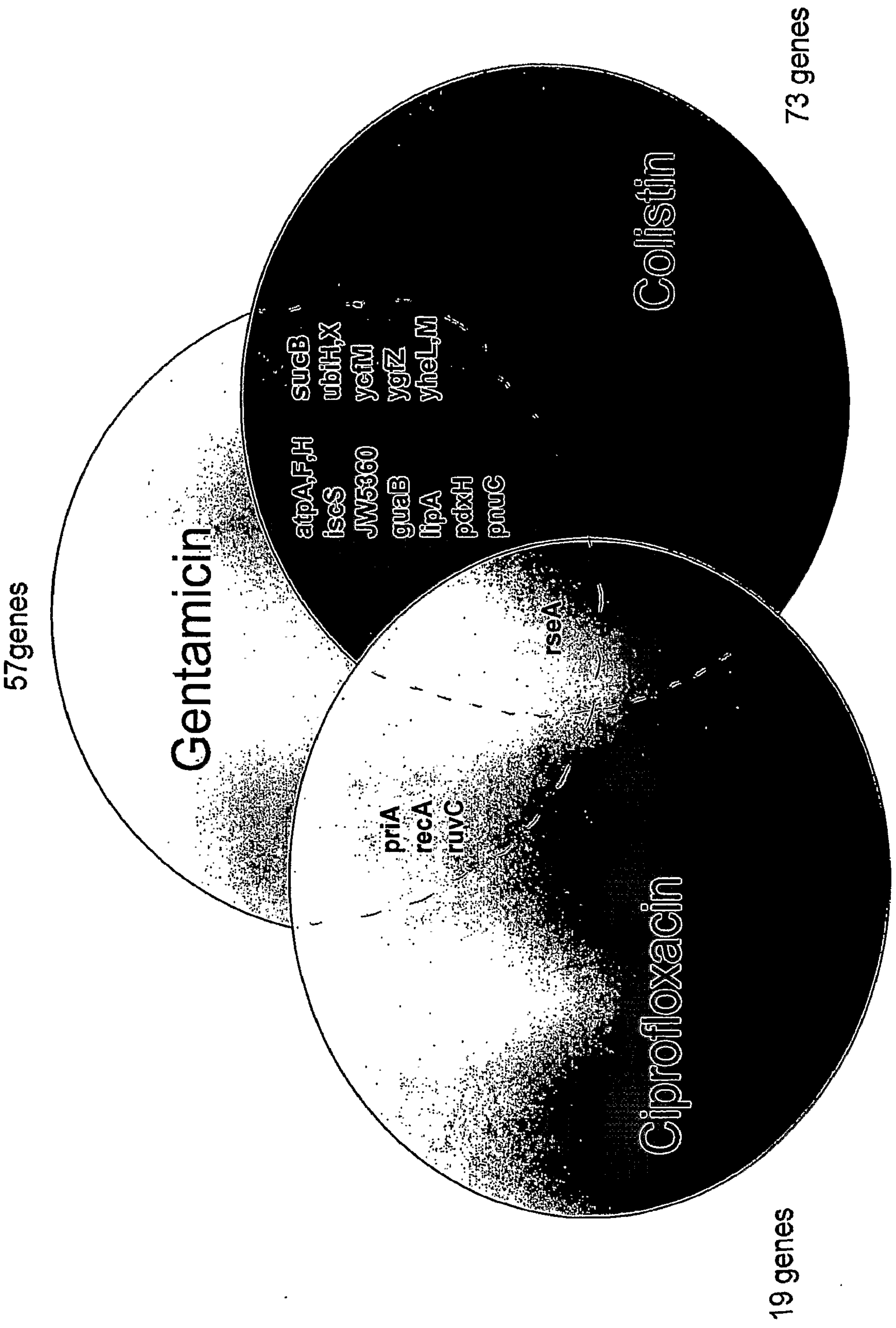

57genes
Gentamicin
atpA,F,H
iscS
JW5360
guaB
lipA
pdxH
pnuC
sucB
ubiH,X
yciM
ygiZ
yheL,M
Collistin
73 genes
rseA
priA
recA
ruvC
Ciprofloxacin
19 genes

Figure 34

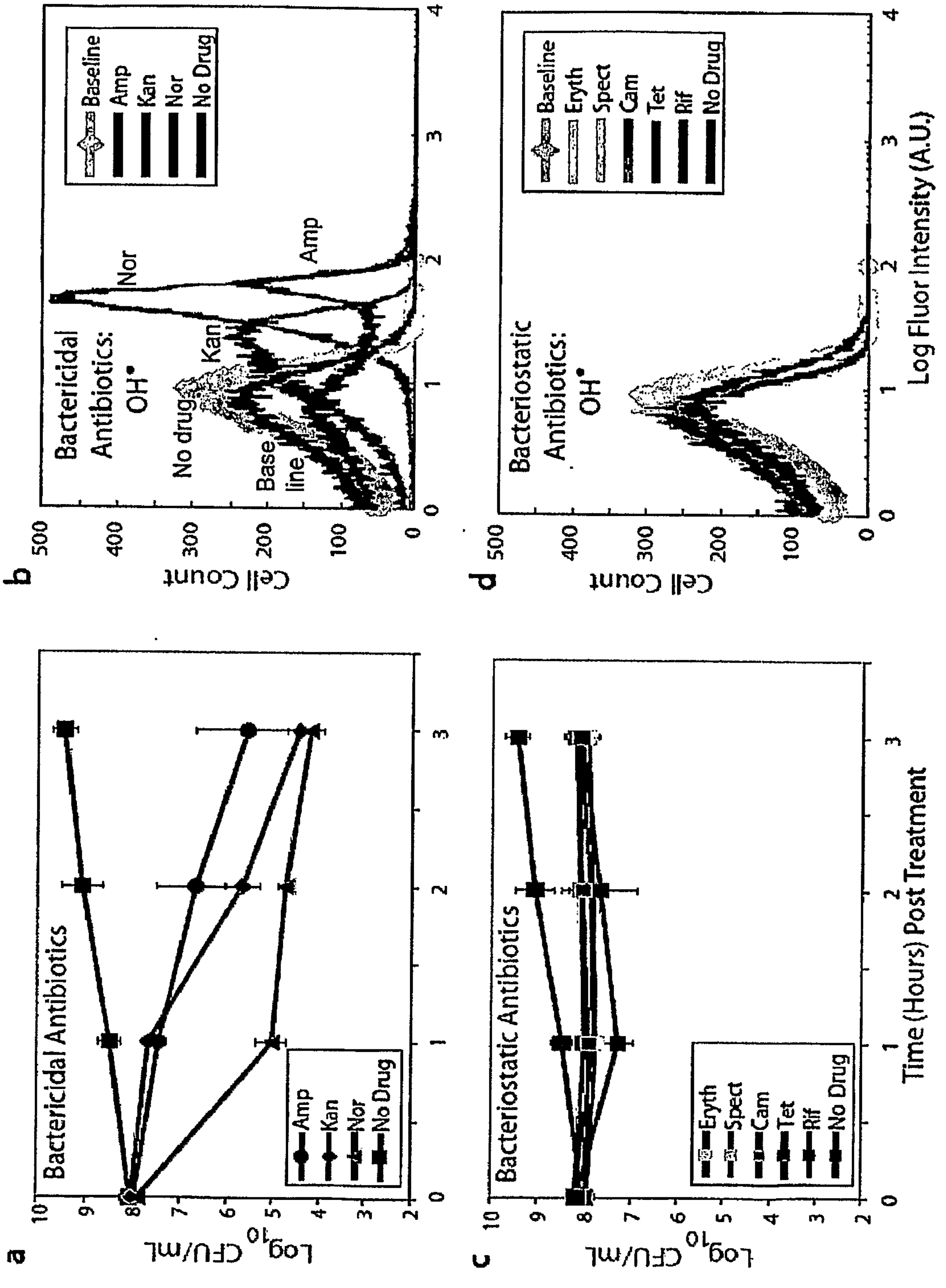

a
Bactericidal Antibiotics
Log$_{10}$ CFU/mL
Amp
Kan
Nor
No Drug
10
9
8
7
6
5
4
3
2
0
1
2
3
b
Bactericidal Antibiotics: OH•
Cell Count
Baseline
Amp
Kan
Nor
No Drug
Nor
Kan
No drug
Base line
Amp
500
400
300
200
100
0
0
1
2
3
4
c
Bacteriostatic Antibiotics
Log$_{10}$ CFU/mL
Eryth
Spect
Cam
Tet
Rif
No Drug
Time (Hours) Post Treatment
d
Bacteriostatic Antibiotics: OH•
Cell Count
Baseline
Eryth
Spect
Cam
Tet
Rif
No Drug
Log Fluor Intensity (A.U.)

Figure 35

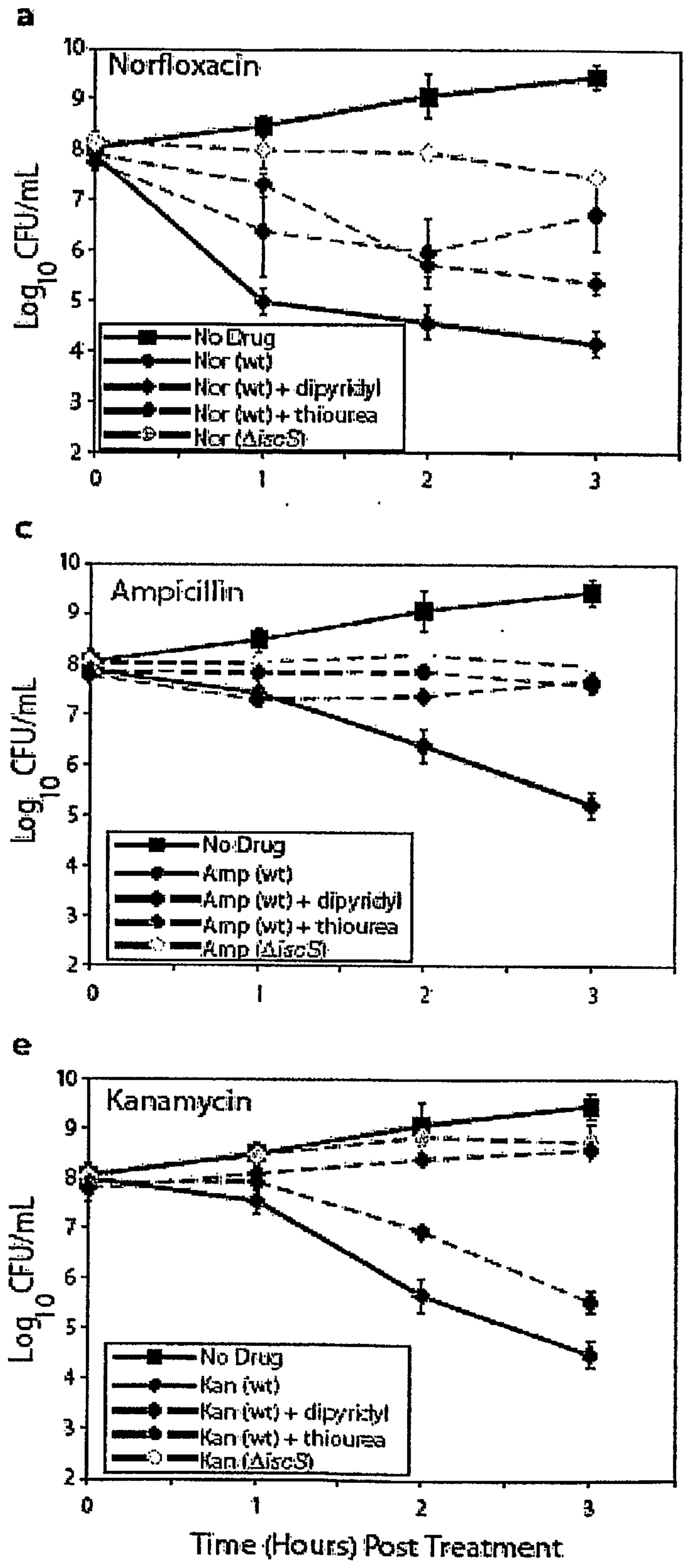

a
Norfloxacin
Log10 CFU/mL
10
9
8
7
6
5
4
3
2
0
1
2
3
No Drug
Nor (wt)
Nor (wt) + dipyridyl
Nor (wt) + thiourea
Nor (ΔiscS)
c
Ampicillin
Log10 CFU/mL
No Drug
Amp (wt)
Amp (wt) + dipyridyl
Amp (wt) + thiourea
Amp (ΔiscS)
e
Kanamycin
Log10 CFU/mL
No Drug
Kan (wt)
Kan (wt) + dipyridyl
Kan (wt) + thiourea
Kan (ΔiscS)
Time (Hours) Post Treatment

Figure 36

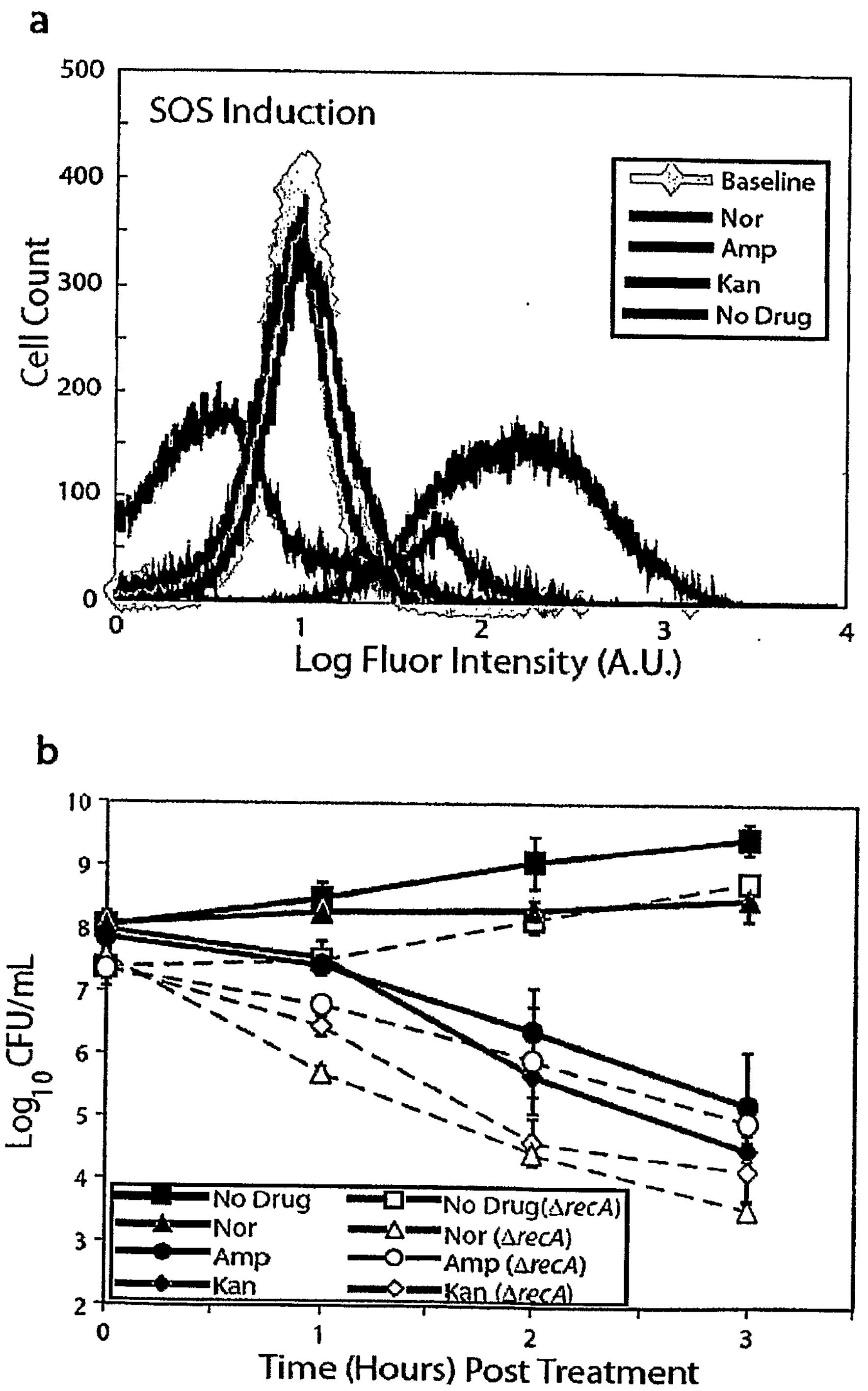

a
SOS Induction
Cell Count
0
100
200
300
400
500
Log Fluor Intensity (A.U.)
0
1
2
3
4
Baseline
Nor
Amp
Kan
No Drug
b
Log_{10}CFU/mL
2
3
4
5
6
7
8
9
10
Time (Hours) Post Treatment
0
1
2
3
No Drug
Nor
Amp
Kan
No Drug(ΔrecA)
Nor (ΔrecA)
Amp (ΔrecA)
Kan (ΔrecA)

Figure 37